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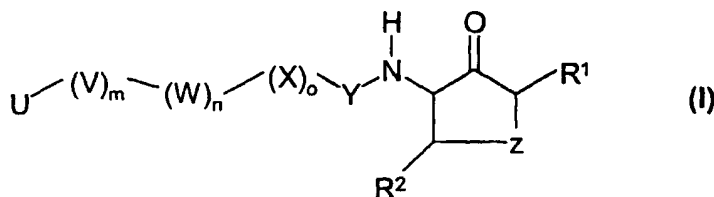
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[Continued on next page]

(54) Title: INHIBITORS OF CRUZIPAIN AND OTHER CYSTEINE PROTEASES



(57) Abstract: Compounds of general formula (I): wherein R¹, R², Y, (X)_o, (W)_n, (V)_m, Z and U are as defined in the specification, are inhibitors of cruzipain and other cysteine protease inhibitors and are useful as therapeutic agents, for example in Chagas' disease, or for validating therapeutic target compounds.



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INHIBITORS OF CRUZIPAIN AND OTHER CYSTEINE PROTEASES

THIS INVENTION relates to compounds which are inhibitors of the protease cruzipain, a gene product of the *Trypanosoma cruzi* parasite. In particular, the invention provides compounds that are useful for the therapeutic treatment of *Trypanosoma cruzi* infection, to the use of these compounds, and to pharmaceutical compositions comprising them. Furthermore, this invention relates to compounds which are inhibitors across a broad range of cysteine proteases, to the use of these compounds, and to pharmaceutical compositions comprising them. Such compounds are useful for the therapeutic treatment of diseases in which participation of a cysteine protease is implicated.

The trypanosomal family of parasites have a substantial worldwide impact on human and animal healthcare (McKerrow, J. H., *et al*, *Ann. Rev. Microbiol.* 47, 821-853, 1993). One parasite of this family, *Trypanosoma cruzi*, is the causative agent of Chagas' disease, which affects in excess of twenty million people annually in Latin and South America, is the leading cause of heart disease in these regions and results in more than 45,000 deaths per annum (Centers for Disease Control and prevention website). In addition, with the increase in migration of the infected population from rural to urban sites and movements from South and Central America into North America, the infection is spreading via blood transfusions, and at birth. The present treatments of choice for *Trypanosoma cruzi* infection, nifurtimox and benznidazole (an NADH fumarate reductase inhibitor, Turrens, JF, *et al*, *Mol Biochem Parasitol.*, 82(1), 125-9, 1996) are at best moderately successful, achieving ~60% cure during the acute phase of infection (see Docampo, R. *Curr. Pharm. Design*, 7, 1157-1164, 2001 for a general discussion) whilst not being prescribed at all during the chronic phase where cardiomyopathy associated heart failure often occurs (Kirchhoff, L. V. *New Engl. J. Med.*, 329, 639-644, 1993). Additionally, these two drugs have serious adverse toxic effects, requiring close medical supervision during treatment, and have been shown to induce chromosomal damage in chagastic infants (Gorla, N. B. *et al*, *Mutat. Res.* 206, 217-220, 1988). Therefore, a strong medical need exists for new effective drugs for the chemotherapeutic treatment of *Trypanosoma cruzi* infection.

Classically, the identification of enzymes found to be crucial for the establishment or propagation of an infectious disease has been instrumental in the development of successful drugs such as antivirals (e.g. HIV aspartyl protease inhibitors) and anti-bacterials (e.g. β -lactam antibiotics). The search for a similar Achilles heel in parasitic infections has examined numerous enzymes (e.g. parasitic dihydrofolate reductase, see Chowdhury, S. F. *et al*, *J. Med. Chem.*, 42(21), 4300-4312, 1999; trypanothione reductase, see Li, Z. *et al*, *Bioorg. Med. Chem. Lett.*, 11(2), 251-254, 2001; parasitic glyceraldehydes-3-phosphate dehydrogenase, see Aranov, A. M. *et al*, *J. Med. Chem.*, 41(24), 4790-4799, 1998). A particularly promising area of research has identified the role of cysteine proteases, encoded by the parasite, that play a pivotal role during the life cycle of the parasite (McKerrow, J. H., *et al*, *Bioorg. Med. Chem.*, 7, 639-644, 1999). Proteases form a substantial group of biological molecules which to date constitute approximately 2% of all the gene products identified following analysis of several genome sequencing programmes (e.g. see Southan, C. *J. Pept. Sci.*, 6, 453-458, 2000). Proteases have evolved to participate in an enormous range of biological processes, mediating their effect by cleavage of peptide amide bonds within the myriad of proteins found in nature. This hydrolytic action is performed by initially recognising, then binding to, particular three-dimensional electronic surfaces displayed by a protein, which aligns the bond for cleavage precisely within the protease catalytic site. Catalytic hydrolysis then commences through nucleophilic attack of the amide bond to be cleaved either via an amino acid side-chain of the protease itself, or through the action of a water molecule that is bound to and activated by the protease. Proteases in which the attacking nucleophile is the thiol side-chain of a Cys residue are known as cysteine proteases. The general classification of 'cysteine protease' contains many members found across a wide range of organisms from viruses, bacteria, protozoa, plants and fungi to mammals.

Biological investigation of *Trypanosoma cruzi* infection has highlighted a number of specific enzymes that are crucial for the progression of the parasite's life cycle. One such enzyme, cruzipain, a cathepsin L-like cysteine protease, is a clear therapeutic

target for the treatment of Chagas' disease ((a) Cazzulo, J. J. *et al*, *Curr. Pharm. Des.* 7, 1143-1156, 2001; (b) Caffrey, C. R. *et al*, *Curr. Drug Targets*, 1, 155-162, 2000). Although the precise biological role of cruzipain within the parasite's life cycle remains unclear, elevated cruzipain messenger RNA levels in the epimastigote
5 developmental stage indicate a role in the nutritional degradation of host-molecules in lysosomal-like vesicles (Engel, J. C. *et al*, *J. Cell. Sci.*, 111, 597-606, 1998). The validation of cruzipain as a viable therapeutic target has been achieved with increasing levels of complexity. Addition of a general cysteine protease inhibitor, Z-Phe-Ala-FMK to *Trypanosoma cruzi*-infected mammalian cell cultures blocked
10 replication and differentiation of the parasite, thus arresting the parasite life cycle (Harth, G., *et al*, *Mol. Biochem. Parasitol.* 58, 17-24, 1993). Administration of a vinyl sulphone-based inhibitor in a *Trypanosoma cruzi*-infected murine animal model not only rescued the mice from lethal infections, but also produced a complete recovery (Engel, J. C. *et al*, *J. Exp. Med.*, 188(4), 725-734, 1998). Numerous other *in*
15 *vivo* studies have confirmed that infections with alternative parasites such as *Leishmania major* (Selzer, P. M. *et al*, *Proc. Nat'l. Acad. Sci. U.S.A.*, 96, 11015-11022, 1999), *Schistosoma mansoni* and *Plasmodium falciparum* (Olson, J. E. *et al*, *Bioorg. Med. Chem.*, 7, 633-638, 1999) can be halted or cured by treatment with cysteine protease inhibitors.

20

A variety of synthetic approaches have been described towards the design of cruzipain inhibitors. However, although providing a biological 'proof-of-principle' for the treatment of *Trypanosoma cruzi* infection, current inhibitors exhibit a number of biochemical and physical properties that may preclude their clinical development.
25 (e.g. see (a) Brinen, L. S. *et al*, *Structure*, 8, 831-840, 2000, peptidomimetic vinyl sulphones, possible adverse mammalian cell toxicity (see McKerrow, J. H. and Engel, J. unpublished results cited in Scheidt, K. A. *et al*, *Bioorg. Med. Chem.*, 6, 2477-2494, 1998); (b) Du, X. *et al*, *Chem. Biol.*, 7, 733-742, 2000, aryl ureas, generally with low μ M activity, and high ClogP values, thus poor aqueous solubility
30 and probably low oral bioavailability; (c) Roush, W. R. *et al*, *Tetrahedron*, 56, 9747-9762, 2000, peptidyl epoxysuccinates, irreversible inhibitors, with potent activity verses house-keeping mammalian proteases such as cathepsin B; (d) Li, R. *et al*,

Bioorg. Med. Chem. 4(9), 1421-1427, **1996**, bisarylacylhydrazides and chalcones, polyhydroxylated aromatics; (e) US6143931, WO 9846559, non-peptide α -ketoamides). Of the many different approaches to enzyme inhibition to date, only the cruzipain protease inhibitors have proven effective in curing disease-related animal
5 models of *Trypanosoma cruzi* infection. Therefore, a clear medical need exists to progress these 'proof-of-principle' findings towards clinical candidates, suitable for human use, through the discovery of more efficacious cruzipain inhibitors that have a desirable combination of potency, selectivity, low toxicity and optimised pharmacokinetic parameters.

10

Cruzipain and indeed many other crucial parasitic proteases belong to the papain-like CA C1 family and have close structural mammalian homologues. Cysteine proteases are classified into 'clans' based upon a similarity in the three-dimensional structure or a conserved arrangement of catalytic residues within the protease primary
15 sequence. Additionally, 'clans' are further classified into 'families' in which each protease shares a statistically significant relationship with other members when comparing the portions of amino acid sequence which constitute the parts responsible for the protease activity (see Barrett, A.J *et al*, in 'Handbook of Proteolytic Enzymes', Eds. Barrett, A. J., Rawlings, N. D., and Woessner, J. F. Publ. Academic
20 Press, **1998**, for a thorough discussion). To date, cysteine proteases have been classified into five clans, CA, CB, CC, CD and CE (Barrett, A. J. *et al*, **1998**). A protease from the tropical papaya fruit 'papain' forms the foundation of clan CA, which currently contains over 80 distinct and complete entries in various sequence databases, with many more expected from the current genome sequencing efforts.
25 Proteases of clan CA/family C1 have been implicated in a multitude of disease processes e.g. human proteases such as cathepsin K (osteoporosis), cathepsin S (autoimmune disorders), cathepsin L (metastases) or parasitic proteases such as falcipain (malaria parasite *Plasmodium falciparum*), cruzipain (*Trypanosoma cruzi* infection). Recently a bacterial protease, staphylopain (*S. aureus* infection) has also
30 been tentatively assigned to clan CA. X-ray crystallographic structures are available for a range of the above mentioned proteases in complex with a range of inhibitors e.g. papain (PDB entries, 1pad, 1pe6, 1pip, 1pop, 4pad, 5pad, 6pad, 1ppp, 1the, 1csb,

1huc), cathepsin K (1au0, 1au2, 1au3, 1au4, 1atk, 1mem, 1bgo, 1ayw, 1ayu), cathepsin L (1cs8), cathepsin S (currently on-hold, but published McGrath, M. E. *et al*, *Protein Science*, 7, 1294-1302, 1998), cruzain (a recombinant form of cruzipain see Eakin, A. E. *et al*, 268(9), 6115-6118, 1993) (1ewp, 1aim, 2aim, 1F29, 1F2A, 5 1F2B, 1F2C), staphylopin (1cv8). Each of the structures displays a similar overall active-site topology, as would be expected by their 'clan' and 'family' classification and such structural similarity exemplifies one aspect of the difficulties involved in discovering a selective inhibitor of cruzipain suitable for human use. However, subtle differences in terms of the depth and intricate shape of the active site groove of each 10 CA C1 protease are evident, which may be exploited for selective inhibitor design. Additionally, many of the current substrate-based inhibitor complexes of CA C1 family proteases show a series of conserved hydrogen bonds between the inhibitor and the protease backbone, which contribute significantly to inhibitor potency. Primarily a bidentate hydrogen-bond is observed between the protease Gly66 (C=O)/ 15 inhibitor N-H and the protease Gly66(NH)/inhibitor (C=O), where the inhibitor (C=O) and (NH) are provided by an amino acid residue NHCHRCO that constitutes the S2 sub-site binding element within the inhibitor (see Berger, A. and Schechter, I. *Philos. Trans. R. Soc. Lond. [Biol.]*, 257, 249-264, 1970 for a description of protease binding site nomenclature). A further hydrogen-bond between the protease main- 20 chain (C=O) of asparagine or aspartic acid (158 to 163, residue number varies between proteases) and an inhibitor (N-H) is often observed, where the inhibitor (N-H) is provided by the S1 sub-site binding element within the inhibitor. Thus, the motif X-NHCHRCO-NH-Y is widely observed amongst the prior art substrate-based inhibitors of CA C1 proteases.

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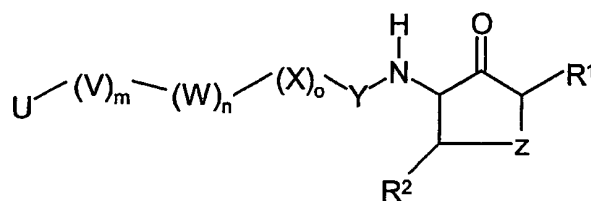
In the prior art, the development of cysteine protease inhibitors for human use has recently been an area of intense activity. Considering the CA C1 family members, particular emphasis has been placed upon the development of inhibitors of human cathepsins, primarily cathepsin K (osteoporosis), cathepsin S (autoimmune disorders) 30 and cathepsin L (metastases), through the use of peptide and peptidomimetic nitriles (e.g. see WO-A-0109910, WO-A-0051998, WO-A-0119816, WO-A-9924460, WO-A-0049008, WO-A-0048992, WO-A-0049007, WO-A-0130772, WO-A-0055125,

WO-A-0055126, WO-A-0119808, WO-A-0149288, WO-A-0147886), linear and cyclic peptide and peptidomimetic ketones (*e.g.* see Veber, D. F. and Thompson, S. K., *Curr. Opin. Drug Discovery Dev.*, 3(4), 362-369, 2000, WO-A-0170232, WO-A-0178734, WO-A-0009653, WO-A-0069855, WO-A-0029408, WO-A-0134153 to
5 WO-A-0134160, WO-A-0029408, WO-A-9964399, WO-A-9805336, WO-A-9850533), ketoheterocycles (*e.g.* see WO-A-0055144, WO-A-0055124) and monobactams (*e.g.* see WO-A-0059881, WO-A-9948911, WO-A-0109169). The prior art describes potent *in vitro* inhibitors, but also highlights the many difficulties in developing a human therapeutic. For example, WO-A-9850533 and WO-A-
10 0029408 describe compounds that may be referred to as cyclic ketones and are inhibitors of cysteine proteases with a particular reference towards papain family proteases and as a most preferred embodiment, cathepsin K. WO-A-9850533 describes compounds subsequently detailed in the literature as potent inhibitors of cathepsin K with good oral bioavailability (Witherington, J., 'Tetrahydrofurans as
15 Selective Cathepsin K Inhibitors', RSC meeting, Burlington House, London, 1999). The compounds of WO-A-9850533 were reported to bind to cathepsin K through the formation of a reversible covalent bond between the tetrahydrofuran carbonyl and the active site catalytic cysteine residue (Witherington, J., 1999). Additionally, the same cyclic ketone compounds are described in WO-A-9953039 as part of a wide-ranging
20 description of inhibitors of cysteine proteases associated with parasitic diseases, with particular reference to the treatment of malaria by inhibition of falcipain. However, subsequent literature describes the cyclic ketone compounds of WO-A-9850533 to be unsuitable for further development or for full pharmacokinetic evaluation due to a physiochemical property of the inhibitors, the poor chiral stability of the α -aminoketone chiral centre (Marquis, R. W. *et al*, *J. Med. Chem.*, 44(5), 725-736,
25 2001). WO-A-0069855 describes compounds that may also be referred to as cyclic ketones with particular reference towards inhibition of cathepsin S. The compounds of WO-A-0069855 are considered to be an advance on compounds of WO-A-9850533 due to the presence of the β -substituent on the cyclic ketone ring system
30 that provides chiral stability to the α -carbon of the cyclic ketone ring system. However, the compounds of WO-A-0069855 and indeed those of WO-A-9850533 describe an absolute requirement for the presence of an amino acid substituent

$R^1R^2NHCHR^3CO-$ that provides the N-terminal portion of the inhibitor molecules, i.e. contain the potential hydrogen-bonding motif $X-NHCHR^3CO-NH-Y$ that is widely observed amongst the prior art substrate-based inhibitors of CA C1 proteases. Although the number of amino acids described both in the literature and commercially available constitute many hundreds of possibilities, in many instances an amino acid backbone may not provide the appropriate balance of properties required for further inhibitor development.

It has now been discovered that certain compounds, defined by general formula (I), are potent and selective cruzipain protease inhibitors which are useful in the treatment of *Trypanosoma cruzi* infection. Other compounds defined by general formula (I) are protease inhibitors across a broad range of CA C1 cysteine proteases and compounds useful in the treatment of diseases caused by cysteine proteases. Compounds described by general formula (I) do not contain the $X-NHCHR^3CO-NH-Y$ motif that is widely observed amongst the prior art substrate-based inhibitors of CA C1 proteases, yet surprisingly compounds defined by general formula (I) retain good potency. The present invention provides substituted (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide, (2-alkyl-4-oxo-tetrahydrothiophen-3-yl) amide and (2-alkyl-5-oxocyclopentyl)amide compounds defined by general formula (I).

Accordingly, the first aspect of the invention provides a compound according to general formula (I):

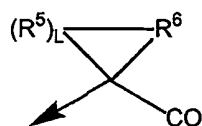


(I)

wherein: $R^1 = C_{0-7}$ -alkyl (when $C = 0$, R^1 is simply hydrogen), C_{3-6} -cycloalkyl or $Ar-C_{0-7}$ -alkyl (when $C = 0$, R^1 is simply an aromatic moiety Ar);

$R^2 = C_{1-7}$ -alkyl, C_{3-6} -cycloalkyl or $Ar-C_{0-7}$ -alkyl;

$Y = \text{CHR}^3\text{-CO}$ or $\text{CR}^3\text{R}^4\text{-CO}$ where R^3 and R^4 are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl and Ar-C_{0-7} -alkyl, or Y represents



5

where L is a number from one to four and R^5 and R^6 are independently chosen from CR^7R^8 where R^7 and R^8 are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl, Ar-C_{0-7} -alkyl or halogen; and for each R^5 and R^6 either R^7 or R^8 (but not both R^7 and R^8) may additionally be chosen from O-C_{0-7} -alkyl, O-C_{3-6} -cycloalkyl, O-Ar-C_{0-7} -alkyl, S-C_{0-7} -alkyl, S-C_{3-6} -cycloalkyl, S-Ar-C_{0-7} -alkyl, NH-C_{0-7} -alkyl, NH-C_{3-6} -cycloalkyl, NH-Ar-C_{0-7} -alkyl, $\text{N-(C}_{0-7}\text{-alkyl)}_2$, $\text{N-(C}_{3-6}\text{-cycloalkyl)}_2$, and $\text{N-(Ar-C}_{0-7}\text{-alkyl)}_2$;

10

$(X)_o = \text{CR}^9\text{R}^{10}$, where R^9 and R^{10} are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl and Ar-C_{0-7} -alkyl and o is a number from zero to three;

15

$(W)_n = \text{O}, \text{S}, \text{C(O)}, \text{S(O)}$ or S(O)_2 or, when n is one or greater, NR^{11} , where R^{11} is chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl and Ar-C_{0-7} -alkyl and n is zero or one;

20

$(V)_m = \text{C(O)}, \text{C(S)}, \text{S(O)}, \text{S(O)}_2, \text{S(O)}_2\text{NH}, \text{OC(O)}, \text{NHC(O)}, \text{NHS(O)}, \text{NHS(O)}_2, \text{OC(O)NH}, \text{C(O)NH}$ or $\text{CR}^{12}\text{R}^{13}$, where R^{12} and R^{13} are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl, Ar-C_{0-7} -alkyl and m is a number from zero to three, provided that when m is greater than one, $(V)_m$ contains a maximum of one carbonyl or sulphonyl group;

25

$Z = \text{O}$ (in which case compounds of general formula (I) may be named as (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amides),

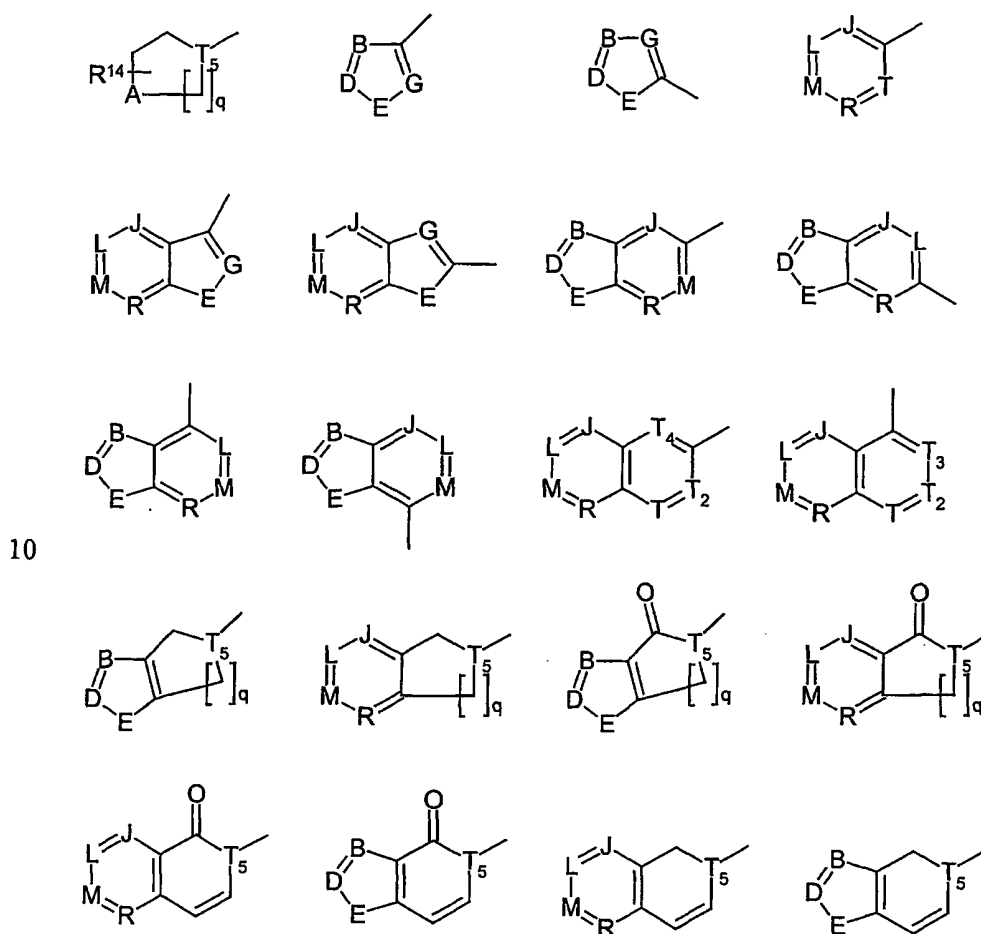
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S (in which case compounds of general formula (I) may be named as (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amides), or

CH₂ (in which case compounds of general formula (I) may be named as (2-alkyl-5-oxocyclopentyl)amides);

5

U = a stable 5- to 7-membered monocyclic or a stable 8- to 11-membered bicyclic ring which is either saturated or unsaturated and which includes zero to four heteroatoms (as detailed below):



wherein R¹⁴ is chosen from:

C₀₋₇-alkyl, C₃₋₆-cycloalkyl, Ar-C₀₋₇-alkyl, halogen, O-C₀₋₇-alkyl, O-C₃₋₆-cycloalkyl, O-Ar-C₀₋₇-alkyl, S-C₀₋₇-alkyl, S-C₃₋₆-cycloalkyl, S-

15

Ar-C₀₋₇-alkyl, NH-C₀₋₇-alkyl, NH-C₃₋₆-cycloalkyl, NH-Ar-C₀₋₇-alkyl, N-(C₀₋₇-alkyl)₂, N-(C₃₋₆-cycloalkyl)₂ and N-(Ar-C₀₋₇-alkyl)₂;

A is chosen from:

5 CH₂, CHR¹⁴, O, S and NR¹⁵, where R¹⁴ is as defined above and where R¹⁵ is chosen from C₀₋₇-alkyl, C₃₋₆-cycloalkyl and Ar-C₀₋₇-alkyl;

B, D and G are independently chosen from:

10 CR¹⁴, where R¹⁴ is as defined above, or N;

E is chosen from:

15 CH₂, CHR¹⁴, O, S and NR¹⁵, where R¹⁴ and R¹⁵ are defined as above ;

J, L, M, R, T, T₂, T₃ and T₄ are independently chosen from:

CR¹⁴ and N, where R¹⁴ is as defined above;

T₅ is chosen from:

20 CH or, only when m + n + o ≥ 1, T₅ may additionally be N;

q is a number from one to three, thereby defining a 5-, 6- or 7-membered ring.

25 B, D, G, J, L, M, R, T, T₂, T₃ and T₄ may additionally represent an N-oxide (N→O).

The present invention includes all salts, hydrates, solvates, complexes and prodrugs of the compounds of this invention. The term "compound" is intended to include all such salts, hydrates, solvates, complexes and prodrugs, unless the context requires
30 otherwise.

Appropriate pharmaceutically and veterinarily acceptable salts of the compounds of general formula (I) include salts of organic acids, especially carboxylic acids, including but not limited to acetate, trifluoroacetate, lactate, gluconate, citrate, tartrate, maleate, malate, pantothenate, adipate, alginate, aspartate, benzoate, butyrate, digluconate, cyclopentanoate, glucoheptanoate, glycerophosphate, oxalate, heptanoate, hexanoate, fumarate, nicotinate, palmoate, pectinate, 3-phenylpropionate, picrate, pivalate, proprionate, tartrate, lactobionate, pivate, camphorate, undecanoate and succinate, organic sulphonic acids such as methanesulphonate, ethanesulphonate, 2-hydroxyethane sulphonate, camphorsulphonate, 2-naphthalenesulphonate, benzenesulphonate, p-chlorobenzenesulphonate and p-toluenesulphonate; and inorganic acids such as hydrochloride, hydrobromide, hydroiodide, sulphate, bisulphate, hemisulphate, thiocyanate, persulphate, phosphoric and sulphonic acids. Salts which are not pharmaceutically or veterinarily acceptable may still be valuable as intermediates.

15

Prodrugs are any covalently bonded compounds which release the active parent drug according to general formula (I) *in vivo*. A prodrug may for example constitute an acetal or hemiacetal derivative of the exocyclic ketone functionality present in the (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide, (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amide and (2-alkyl-5-oxocyclopentyl)amide scaffold. If a chiral centre or another form of isomeric centre is present in a compound of the present invention, all forms of such isomer or isomers, including enantiomers and diastereoisomers, are intended to be covered herein. Compounds of the invention containing a chiral centre may be used as a racemic mixture, an enantiomerically enriched mixture, or the racemic mixture may be separated using well-known techniques and an individual enantiomer may be used alone.

25

'Halogen' as applied herein is meant to include F, Cl, Br, I;

30 'Heteroatom' as applied herein is meant to include O, S and N;

'C₀₋₇-alkyl' as applied herein is meant to include stable straight and branched chain aliphatic carbon chains containing zero (*i.e.* simply hydrogen) to seven carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, pentyl, isopentyl, hexyl, heptyl and any simple isomers thereof. Additionally, any C₀₋₇-alkyl
5 may optionally be substituted at any point by one, two or three halogen atoms (as defined above) for example to give a trifluoromethyl substituent. Furthermore, C₀₋₇-alkyl may contain one or more heteroatoms (as defined above) for example to give ethers, thioethers, sulphones, sulphonamides, substituted amines, amidines, guanidines, carboxylic acids, carboxamides. If the heteroatom is located at a chain
10 terminus then it is appropriately substituted with one or two hydrogen atoms. A heteroatom or halogen is only present when C₀₋₇-alkyl contains a minimum of one carbon atom.

C₁₋₇-alkyl as applied herein is meant to include the definitions for C₀₋₇-alkyl (as
15 defined above) but describes a substituent that comprises a minimum of one carbon.

'C₃₋₆-cycloalkyl' as applied herein is meant to include any variation of 'C₀₋₇-alkyl' which additionally contains a carbocyclic ring such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl. The carbocyclic ring may optionally be substituted with one
20 or more halogens (as defined above) or heteroatoms (as defined above) for example to give a tetrahydrofuran, pyrrolidine, piperidine, piperazine or morpholine substituent.

'Ar-C₀₋₇-alkyl' as applied herein is meant to include any variation of C₀₋₇-alkyl which
25 additionally contains an aromatic ring moiety 'Ar'. The aromatic ring moiety Ar can be a stable 5 or 6-membered monocyclic or a stable 9 or 10 membered bicyclic ring which is unsaturated, as defined previously for U in general formula (I). The aromatic ring moiety Ar may be substituted by R¹⁴ (as defined above for U in general formula (I)). When C = 0 in the substituent Ar-C₀₋₇-alkyl, the substituent is simply
30 the aromatic ring moiety Ar.

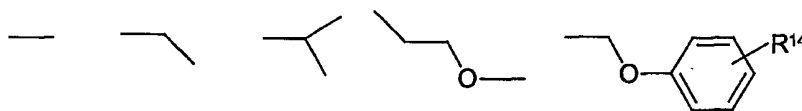
Other expressions containing terms such as alkyl and cycloalkyl are intended to be construed according to the definitions above. For example "C₁₋₄ alkyl" is the same as C₀₋₇-alkyl except that it contains from one to four carbon atoms.

- 5 If different structural isomers are present, and/or one or more chiral centres are present, all isomeric forms are intended to be covered. Enantiomers are characterised by the absolute configuration of their chiral centres and described by the *R*- and *S*-sequencing rules of Cahn, Ingold and Prelog. Such conventions are well known in the art (e.g. see 'Advanced Organic Chemistry', 3rd edition, ed. March, J., John Wiley and Sons, New York, 1985).

Preferred compounds of general formula (I) include those in which R¹ comprises C₀₋₇-alkyl or Ar-C₀₋₇-alkyl. Thus, for example, preferred R¹ moieties include hydrogen, or a straight or branched alkyl chain, or a straight or branched heteroalkyl chain, or an optionally substituted arylalkyl chain, or an optionally substituted arylheteroalkyl chain.

It is particularly preferred that R¹ is hydrogen or C₁₋₄ alkyl or Ar-C₁₋₄-alkyl and examples of such R¹ substituents include, but are not limited to:

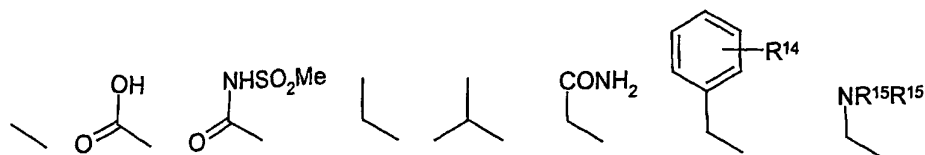
20



It is preferred that R² is C₁₋₇-alkyl or Ar-C₀₋₇-alkyl, for example, straight or branched alkyl chains or heteroalkyl chains or optionally substituted aralkyl chains or an alkylcarboxylic ester chain or an N-(alkylcarbonyl)sulphonamide chain or an alkyl carboxamide chain.

25

Particularly preferred compounds include those in which R² is C₁₋₄-alkyl or Ar-C₁₋₄-alkyl and examples of such R² substituents include, but are not limited to:

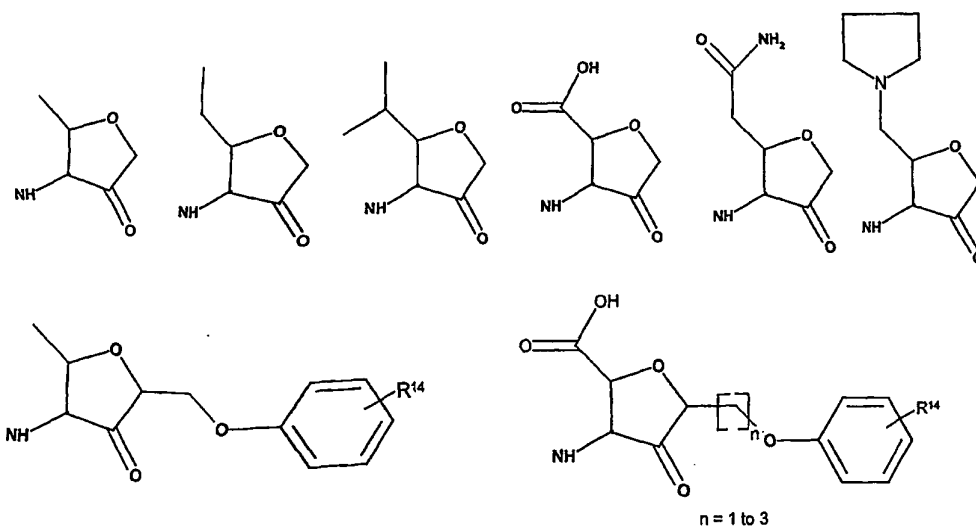


wherein R^{14} and R^{15} are as defined previously.

- 5 Additionally, preferred compounds of general formula (I) comprise those in which Z = 'O'.

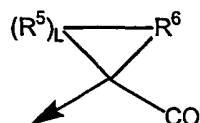
Combining the preferred examples of the substituents R^1 and R^2 with an exemplary (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide scaffold yields the following compounds,

- 10 which are among those preferred:



- 15 In preferred compounds of general formula (I), Y is CHR^4CO where R^4 is selected from C_{0-7} -alkyl or $Ar-C_{0-7}$ -alkyl, for example hydrogen, a straight or branched alkyl chain, a straight or branched heteroalkyl chain, an optionally substituted arylalkyl chain or an optionally substituted arylheteroalkyl chain. Additionally in preferred compounds of general formula (I), R^4 is selected from C_{3-6} -cycloalkyl, for example cyclohexylmethyl or cyclopentylmethyl.

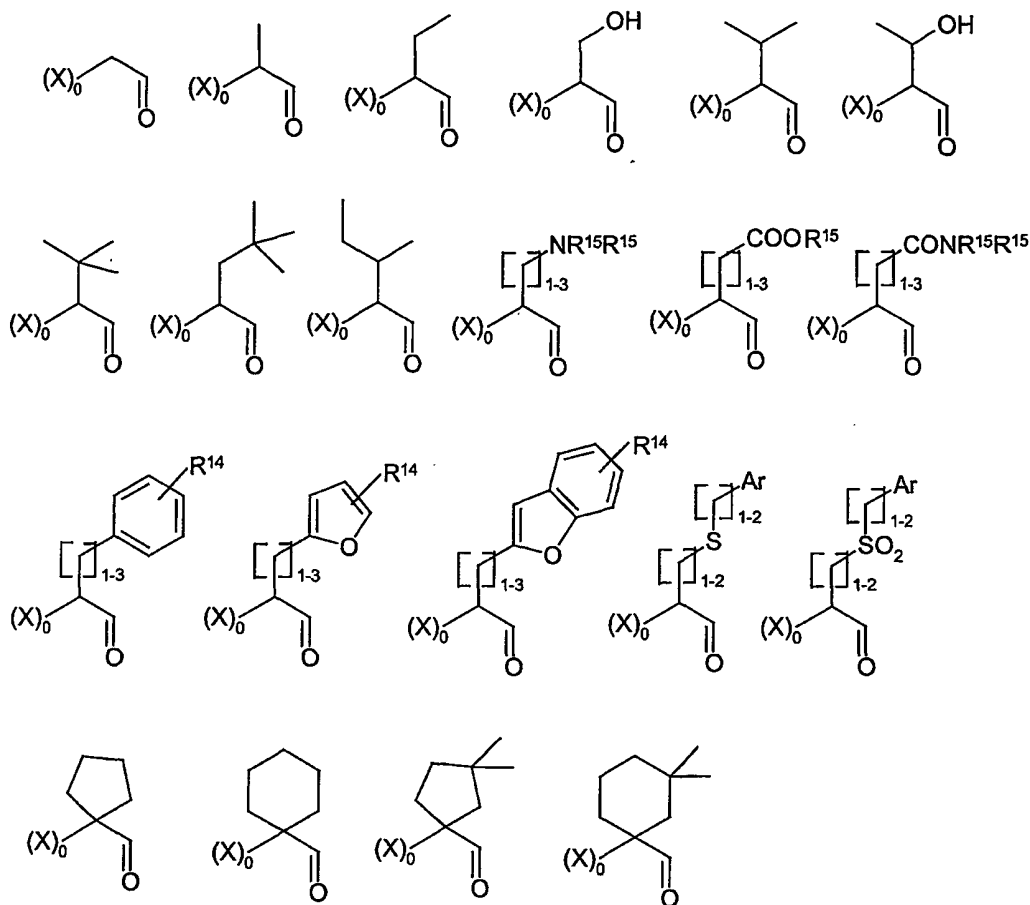
Other preferred compounds of general formula (I) are those in which Y comprises a group:



- 5 where R^5 and R^6 are each CR^7R^8 and each R^7 and R^8 is, independently, selected from C_{0-7} -alkyl or $Ar-C_{0-7}$ -alkyl, for example hydrogen, a straight or branched alkyl chain, a straight or branched heteroalkyl chain, an optionally substituted arylalkyl chain or an optionally substituted arylheteroalkyl chain.
- 10 More preferred R^7 and R^8 groups include C_{0-4} alkyl, for example hydrogen, methyl and ethyl.

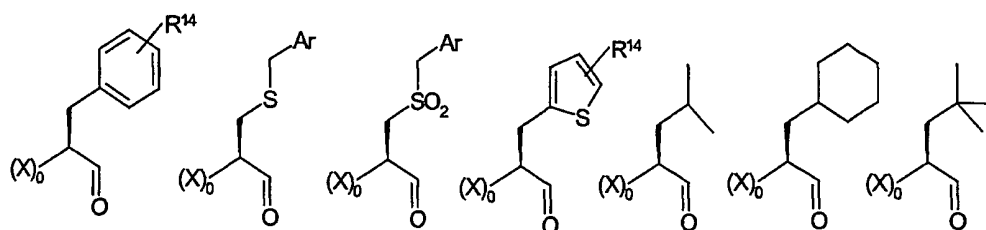
Examples of preferred Y substituents include the following:

-16-



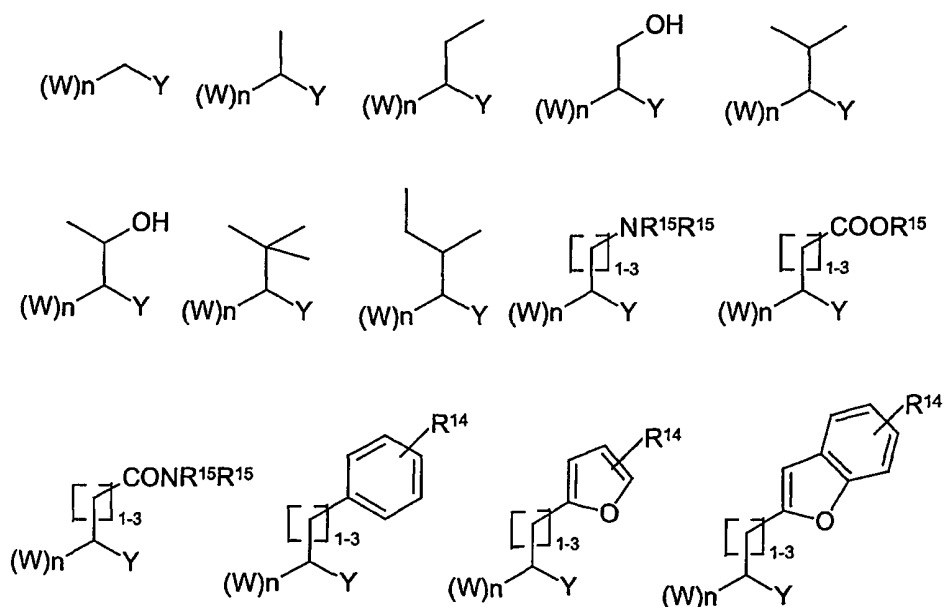
wherein R^{14} and R^{15} and Ar are as defined previously.

- 5 More preferred compounds of general formula (I), comprise an R^4 group chosen from C_{1-4} -alkyl, which may be substituted with OH, $NR^{15}R^{15}$, $COOR^{15}$, or $CONR^{15}$; or Ar- C_{1-4} -alkyl, where the aryl group may be substituted with R^{14} ; wherein each R^{14} and R^{15} is independently as defined above.
- 10 Even more preferred R^4 groups comprise Ar- CH_2 -, where the aromatic ring is an optionally substituted phenyl or monocyclic heterocycle. Additionally, even more preferred R^4 groups comprise simple branched alkyl groups such as isobutyl or straight heteroalkyl chains such as benzylsulfanylmethyl or benzylsulphonylmethyl. Furthermore, even more preferred R^4 groups comprise cyclohexylmethyl. Examples
- 15 of even more preferred Y substituents comprise the following,



wherein R^{14} and Ar are as defined previously

- 5 It is preferred that in the group $(X)_0$, each of R^9 and R^{10} is selected from C_{0-7} -alkyl or Ar- C_{0-7} -alkyl, for example hydrogen, a straight or branched alkyl chain, a straight or branched heteroalkyl chain, an optionally substituted arylalkyl chain or an optionally substituted arylheteroalkyl chain.
- 10 More preferred $(X)_0$ groups comprise R^9 chosen from hydrogen; R^{10} is C_{1-4} -alkyl, which may be substituted with OH, $NR^{15}R^{15}$, $COOR^{15}$, or $CONR^{15}$; or Ar- C_{1-4} -alkyl, where the aryl group may be substituted with R^{14} , wherein each R^{14} and R^{15} is independently as defined above.
- 15 Examples of preferred $(X)_0$ groups include the following:



-18-

wherein R^{14} and R^{15} are as defined previously.

Even more preferred compounds of general formula (I), comprise $(X)_o$ groups that are simple alkyl groups such as methylene and where $o = 0$ or 1 .

5

In the group $(W)_n$:

W is preferably O, S, SO_2 , S(O), C(O) or when o is one or greater, NR^{11} , where R^{11} is C_{0-4} -alkyl; and n is 0 or 1.

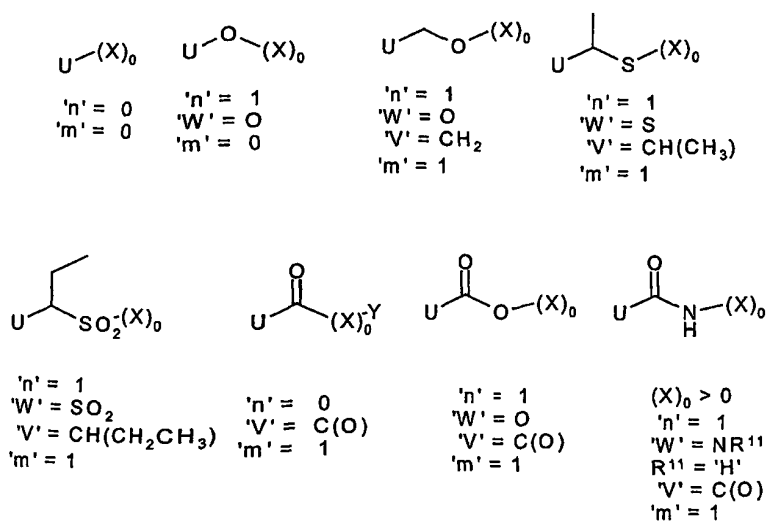
- 10 More preferred compounds of general formula (I), comprise $(W)_n$ groups defined as O, S, SO_2 , C(O) and where $n = 0$ or 1 .

In the group $(V)_m$:

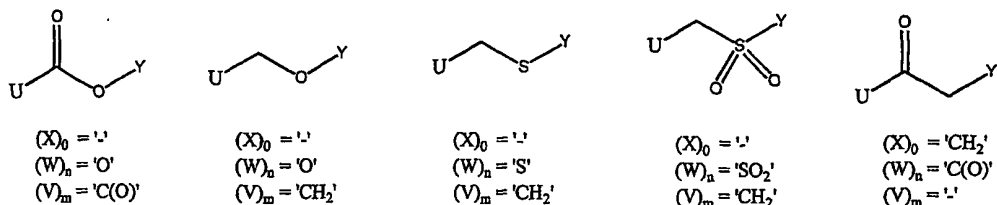
V is preferably C(O), C(O)NH or CHR^{13} , where R^{13} is C_{0-4} -alkyl; and

- 15 m is 0 or 1.

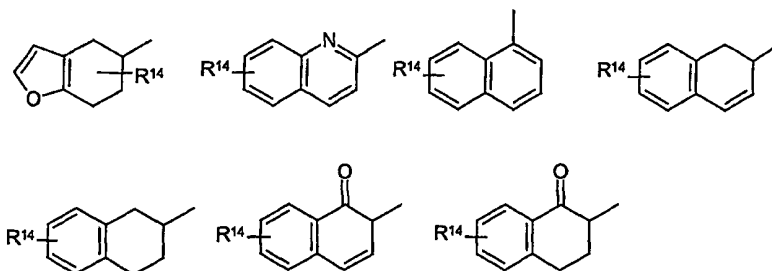
Preferred V and W substituent combinations encompassed by general formula (I) include, but are not limited to:



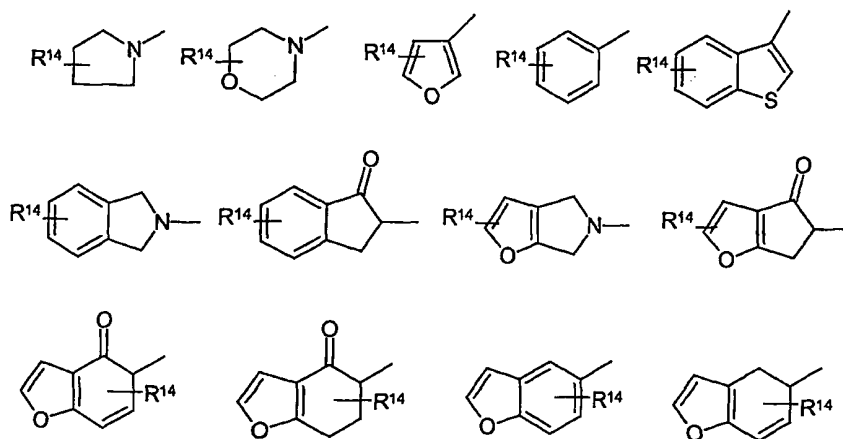
More preferred V, W and X substituent combinations encompassed by general formula (I) comprise, but are not limited to



- 5 In preferred compounds of general formula (I), U comprises an optionally substituted 5- or 6-membered saturated or unsaturated heterocycle or Ar group or an optionally substituted saturated or unsaturated 9- or 10-membered heterocycle or Ar group. Examples of such preferred U rings include the following:

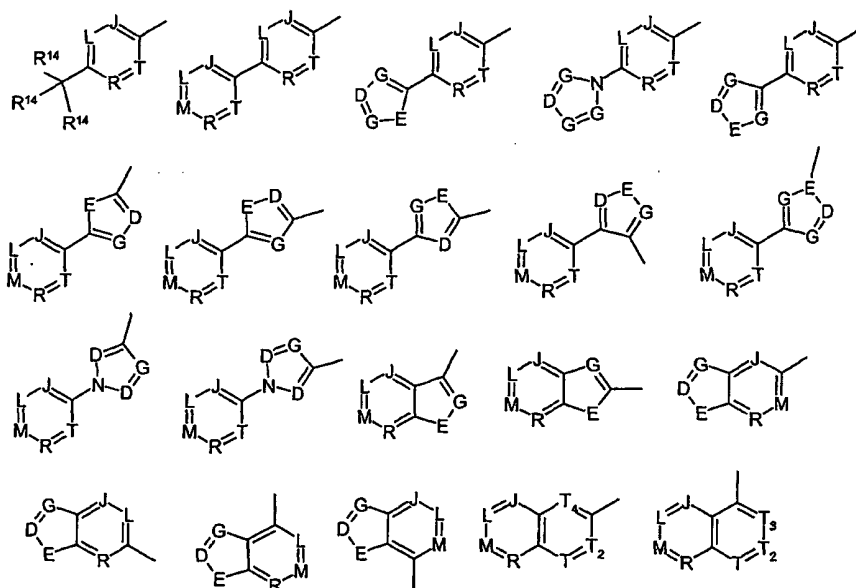


- 10 and also the following



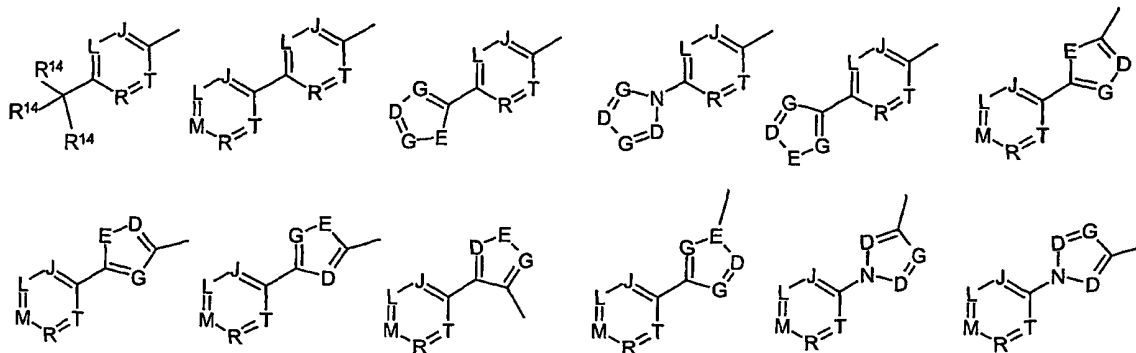
wherein R^{14} is as defined previously.

- More preferred compounds of general formula (I), contain a U group comprising of a bulky alkyl or aryl group at the para position of an aryl Ar. Also, more preferred compounds contain a meta or para-biaryl Ar-Ar, where Ar is as previously defined. Additionally, more preferred compounds contain a 6,6 or 6,5 or 5,6-fused aromatic ring. Examples of more preferred U groups are



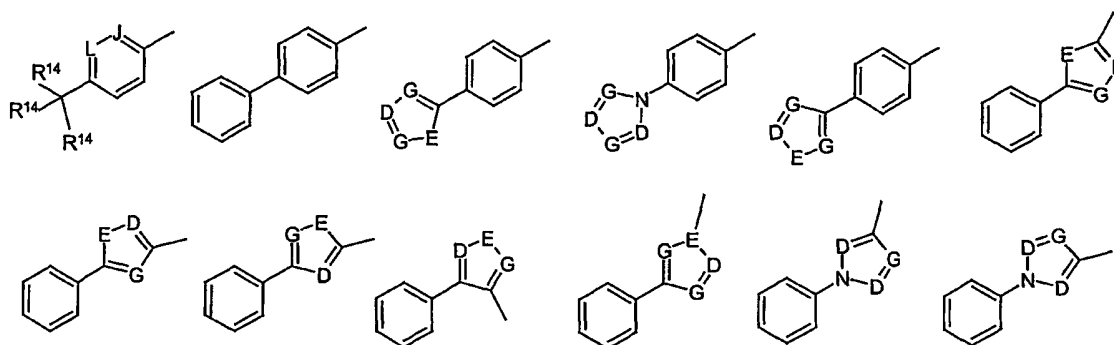
wherein R^{14} , D, E, G, J, L, M, R, T, T_2 , T_3 and T_4 are as defined previously.

- Even more preferred compounds of general formula (I), particularly for inhibition of cruzipain, contain a U group comprising a 6-membered Ar ring containing a bulky alkyl or aryl group at the para position, where Ar is as previously defined



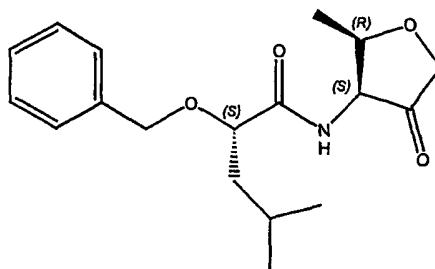
wherein R^{14} , D, E, G, J, L, M, R and T are as defined previously

Yet even more preferred compounds of general formula (I), contain a U group
5 comprising but are not limited to the following,



wherein R^{14} , D, E, G, J and L are as defined previously.

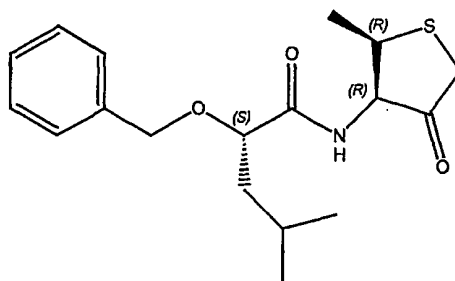
- 10 Abbreviations and symbols commonly used in the peptide and chemical arts are used herein to describe compounds of the present invention, following the general guidelines presented by the IUPAC-IUB Joint Commission on Biochemical Nomenclature as described in *Eur. J. Biochem.*, 158, 9-, 1984. Compounds of formula (I) and the intermediates and starting materials used in their preparation are
15 named in accordance with the IUPAC rules of nomenclature in which the characteristic groups have decreasing priority for citation as the principle group. An example compound of formula (I), compound (1) in which R^1 is H, R^2 is methyl, Z is oxygen, Y is 4-methylpentyl, $(X)_0$ is zero, $(W)_n$ is oxygen, $(V)_m$ is methylene and U is phenyl is thus named:-



(1)

2*S*-Benzyloxy-4-methyl-pentanoic acid (2*R*-methyl-4-oxo-tetrahydrofuran-3*S*-yl)-amide

- 5 A second example compound of formula (I), compound (2) in which R^1 is H, R^2 is methyl, Z is sulphur, Y is 4-methylpentyl, $(X)_0$ is zero, $(W)_n$ is oxygen, $(V)_m$ is methylene and U is phenyl is thus named:-

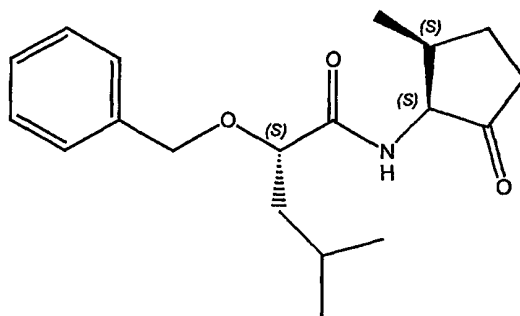


10

(2)

2*S*-Benzyloxy-4-methyl-pentanoic acid (2*R*-methyl-4-oxo-tetrahydrothiophen-3*R*-yl)amide

- 15 A third example compound of formula (I), compound (3) in which R^1 is H, R^2 is methyl, Z is methylene, Y is 4-methylpentyl, $(X)_0$ is zero, $(W)_n$ is oxygen, $(V)_m$ is methylene and U is phenyl is thus named:-



(3)

- 20 2*S*-Benzyloxy-4-methylpentanoic acid (2*S*-methyl-5-oxo-cyclopent-3*S*-yl)amide

Compounds of the invention include, but are not limited to, the following examples where all 4 stereoisomeric combinations of the cyclic ketone are included, i.e. (2*S*,

3*S*), (2*R*, 3*S*), (2*S*, 3*R*), (2*R*, 3*R*) and where Z = 'O' and R¹ = 'H', and also include the equivalent analogues included in the full definition of Z and R¹ and R²

4-Methyl-2-(4-trifluoromethylbenzyloxy)-pentanoic acid (2-methyl-4-oxo-tetrahydro-
5 furan-3-yl)-amide

N-(2-Methyl-4-oxo-tetrahydro-furan-3-yl)-3-phenyl-2-(4-trifluoromethylbenzyloxy)-
propionamide

10 3-(4-Hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(4-
trifluoromethyl-benzyloxy)-propionamide

2-Benzyloxy-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

15 2-Benzyloxy-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-3-phenyl-propionamide

2-Benzyloxy-3-(4-hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-
propionamide

20 4-Methyl-2-(4-thiophen-2-yl-benzyloxy)-pentanoic acid (2-methyl-4-oxo-tetrahydro-
furan-3-yl)-amide

N-(2-Methyl-4-oxo-tetrahydro-furan-3-yl)-3-phenyl-2-(4-thiophen-2-yl-benzyloxy)-
propionamide

25 3-(4-Hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(4-thiophen-2-yl-
benzyloxy)-propionamide

2-(4-*tert*-Butyl-benzyloxy)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-
30 furan-3-yl)-amide

2-(4-*tert*-Butyl-benzyloxy)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-3-phenyl-
propionamide

- 2-(4-*tert*-Butyl-benzyloxy)-3-(4-hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 5 2-(Biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 2-(Biphenyl-4-ylmethoxy)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-3-phenyl-propionamide
- 10 2-(Biphenyl-4-ylmethoxy)-3-(4-hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 2-(4-*tert*-Butyl-benzylsulfanyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 15 2-(4-*tert*-Butyl-benzylsulfanyl)-3-(4-hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 20 2-(Biphenyl-4-ylmethysulfanyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 2-(Biphenyl-4-ylmethysulfanyl)-3-(4-hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 25 4-Methyl-2-(4-thiophen-2-yl-benzylsulfanyl)-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 30 3-(4-Hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(4-thiophen-2-yl-benzylsulfanyl)-propionamide

2-(4-*tert*-Butyl-phenylmethanesulfonyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

2-(4-*tert*-Butyl-phenylmethanesulfonyl)-3-(4-hydroxyphenyl)-*N*-(2-methyl-4-oxo-
5 tetrahydro-furan-3-yl)-propionamide

2-(Biphenyl-4-ylmethanesulfonyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

10 2-(Biphenyl-4-ylmethanesulfonyl)-3-(4-hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide

4-Methyl-2-(4-thiophen-2-yl-phenylmethanesulfonyl)-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

15 3-(4-Hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(4-thiophen-2-yl-phenylmethanesulfonyl)-propionamide

3-Phenyl-pyrrole-1-carboxylic acid 2-(4-hydroxyphenyl)-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester
20

3-Phenyl-pyrrole-1-carboxylic acid 3-methyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-butyl ester

25 1,3-Dihydro-isoindole-2-carboxylic acid 2-(4-hydroxyphenyl)-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester

1,3-Dihydro-isoindole-2-carboxylic acid 3-methyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-butyl ester

30 3,4-Dihydro-1*H*-isoquinoline-2-carboxylic acid 2-(4-hydroxyphenyl)-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester

3,4-Dihydro-1*H*-isoquinoline-2-carboxylic acid 3-methyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-butyl ester

- 5 2-(4-Hydroxybenzyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-4-oxo-4-(3-phenyl-pyrrol-1-yl)-butyramide

4-Methyl-2-[2-oxo-2-(3-phenyl-pyrrol-1-yl)-ethyl]-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

10

4-(1,3-Dihydro-isoindol-2-yl)-2-(4-hydroxybenzyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-4-oxo-butyramide

- 2-[2-(1,3-Dihydro-isoindol-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 15

4-(3,4-Dihydro-1*H*-isoquinolin-2-yl)-2-(4-hydroxybenzyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-4-oxo-butyramide

- 20 2-[2-(3,4-Dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

- Additional compounds of the invention include, but are not limited to, the following examples where all 4 stereoisomeric combinations of the cyclic ketone are included, i.e. (2*S*, 3*S*), (2*R*, 3*S*), (2*S*, 3*R*), (2*R*, 3*R*) and where Z = 'O' and R¹ = 'H', and also include the equivalent analogues included in the full definition of Z and R¹ and R²
- 25

3-(4-Hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(naphthalen-1-ylmethoxy)-propionamide

30

N-(2-Methyl-4-oxo-tetrahydro-furan-3-yl)-2-(naphthalen-1-ylmethoxy)-3-phenyl-propionamide

- 2-Benzoyloxy-3-cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 3-Cyclohexyl-2-(furan-3-ylmethoxy)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-
5 propionamide
- 3-Cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(thiophen-3-ylmethoxy)-
propionamide
- 10 3-Cyclohexyl-2-(furan-2-ylmethoxy)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-
propionamide
- 3-Cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(thiophen-2-ylmethoxy)-
propionamide
15
- 2-(Benzo[b]thiophen-3-ylmethoxy)-3-cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro-
furan-3-yl)-propionamide
- 2-(Furan-3-ylmethoxy)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-
20 furan-3-yl)-amide
- 4,4-Dimethyl-2-(thiophen-3-ylmethoxy)-pentanoic acid (2-methyl-4-oxo-tetrahydro-
furan-3-yl)-amide
- 25 2-(Furan-2-ylmethoxy)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-
furan-3-yl)-amide
- 4,4-Dimethyl-2-(thiophen-2-ylmethoxy)-pentanoic acid (2-methyl-4-oxo-tetrahydro-
furan-3-yl)-amide
30
- 2-(Benzo[b]thiophen-3-ylmethoxy)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-
tetrahydro-furan-3-yl)-amide

- 3-Cyclohexyl-2-(furan-3-ylmethylsulfanyl)-N-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 5 3-Cyclohexyl-N-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(thiophen-3-ylmethylsulfanyl)-propionamide
- 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-N-(2-methyl-4-oxo-tetrahydro-furan-2-yl)-propionamide
- 10 3-Cyclohexyl-N-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(thiophen-2-ylmethylsulfanyl)-propionamide
- 15 2-(Benzo[b]thiophen-3-ylmethylsulfanyl)-3-cyclohexyl-N-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 2-(Furan-3-ylmethylsulfanyl)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 20 4,4-Dimethyl-2-(thiophen-3-ylmethylsulfanyl)-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 2-(Furan-2-ylmethylsulfanyl)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 25 4,4-Dimethyl-2-(thiophen-2-ylmethylsulfanyl)-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 2-(Benzo[b]thiophen-3-ylmethylsulfanyl)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide .
- 30

- 3-(4-Hydroxyphenyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(naphthalen-1-ylmethylsulfonyl)-propionamide
- 3-Cyclohexyl-2-(furan-3-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 5
- 3-Cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(thiophen-3-ylmethanesulfonyl)-propionamide
- 10
- 3-Cyclohexyl-2-(furan-2-ylmethylsulfonyl)-*N*-(2-methyl-4-oxo-tetrahydro-furan-2-yl)-propionamide
- 3-Cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(thiophen-2-ylmethylsulfonyl)-propionamide
- 15
- 2-(Benzo[b]thiophen-3-ylmethylsulfonyl)-3-cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide
- 2-(Furan-3-ylmethylsulfonyl)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 20
- 4,4-Dimethyl-2-(thiophen-3-ylmethylsulfonyl)-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 2-(Furan-2-ylmethylsulfonyl)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 25
- 4,4-Dimethyl-2-(thiophen-2-ylmethylsulfonyl)-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide
- 30
- 2-(Benzo[b]thiophen-3-ylmethylsulfonyl)-4,4-dimethyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

3-(4-Hydroxyphenyl)-N-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-2-(naphthalen-1-ylmethylsulfonyl)-propionamide

- 5 Morpholine-4-carboxylic acid 2-cyclohexyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester

Morpholine-4-carboxylic acid 3,3-dimethyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-butyl ester

10

2-Cyclohexylmethyl-N-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-4-morpholin-4-yl-4-oxo-butyramide

- 15 4,4-Dimethyl-2-(2-morpholin-4-yl-2-oxo-ethyl)-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

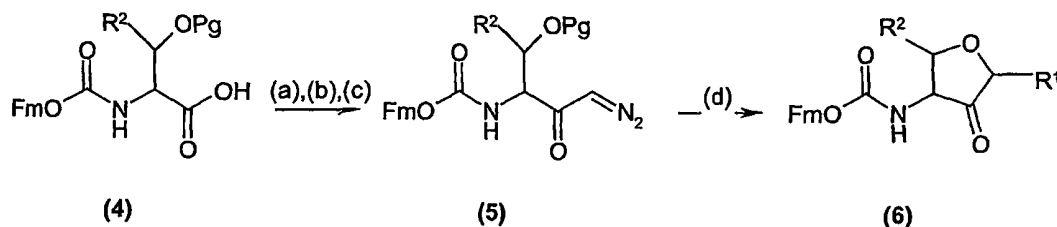
2-Biphenyl-3-yl-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide

- 20 To those skilled in the practices of organic chemistry, compounds of general formula (I) may be readily synthesised by a number of chemical strategies, performed either in solution or on the solid phase (see Atherton, E. and Sheppard, R. C. In '*Solid Phase Peptide Synthesis: A Practical Approach*', Oxford University Press, Oxford, U.K. 1989, for a general review of solid phase synthesis principles). The solid phase
25 strategy is attractive in being able to generate many thousands of analogues, typically on a 5-100mg scale, through established parallel synthesis methodologies (e.g. see (a) Bastos, M.; Maeji, N. J.; Abeles, R. H. *Proc. Natl. Acad. Sci. USA*, 92, 6738-6742, 1995).

- 30 Therefore, one strategy for the synthesis of compounds of general formula (I) comprises:-

- (a) Preparation of an appropriately functionalised and protected (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide or a (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amide or a (2-alkyl-5-oxocyclopentyl)amide building block in solution.
- (b) Attachment of the building block (a) to the solid phase through a linker that is stable to the conditions of synthesis but readily labile to cleavage at the end of a synthesis (see James, I. W., *Tetrahedron*, 55(Report N^o 489), 4855-4946, 1999, for examples of the 'linker' function as applied to solid phase synthesis).
- (c) Solid phase organic chemistry (see Brown, R. D. *J. Chem. Soc., Perkin Trans. 1*, 19, 3293-3320, 1998), to construct the remainder of the molecule.
- (d) Compound cleavage from the solid phase into solution.
- (e) Cleavage work-up and compound analysis.

The first stage in a synthesis of compounds of general formula (I) is the preparation in solution of a functionalised and protected building block. A typical scheme towards the (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide (6) is detailed in Scheme 1.



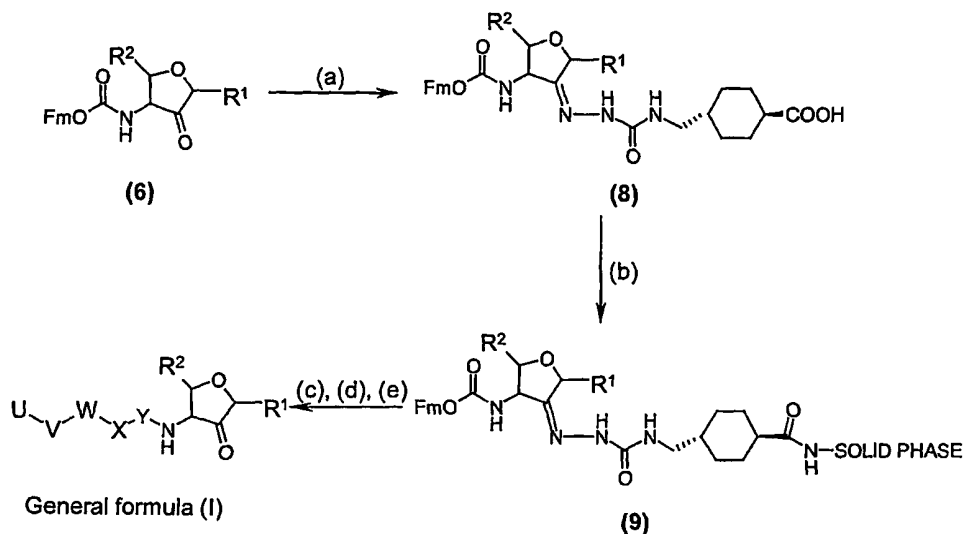
Scheme 1. (a) ⁱBuOCCl, NMM, DCM, -15°C, 10mins, under argon. (b) Diazomethane in diethyl ether, -15°C to RT over 1hr. (c) Acetic acid (d) LiCl (10eq) in 80%aq acetic acid, 5°C to RT over 1hr.

- Fmoc(O) denotes the well known amine protecting group 9-fluorenyl methoxycarbonyl (Fmoc, see Atherton, E. and Sheppard, R. C., 1989) and 'Pg' denotes either a free hydroxyl or an hydroxyl protecting group such as *tert*-butyl ether. In the illustrated case, condensation with diazomethane provides R¹ = H.
- Considering step (a), synthesis may commence from suitably protected β-hydroxy-α-amino acids (4), which are accessible through a variety of literature methods e.g. (a) Adams, Z. M., Jackson, R. F. W., Palmer, N. J., Rami, H. K., Wythes, M. J., *J.*

Chem. Soc., Perkin Trans I, 937-947, 1999, (b) Hubschwerlen, C., *et al*, *J. Med. Chem.*, 41, 3972-3975, 1998, (c) Luzzio, F. A., *et al*, *Tet. Lett.*, 41, 7151-7155, 2000, (d) Morgan, A. J. *et al*, *Org. Lett.* 1(12), 1949-1952, 1999. (e) Zhang, H., Xia, P., Zhou, W., *Tetrahedron: Asymmetry*, 11, 3439-3447, 2000, (f) Blaskovich, M.A., *et al*, *J. Org. Chem.*, 63, 3631-3646, 1998.

In the simplest case where R² is a methyl substituent, the β-hydroxy-α-amino acid (4) is threonine (Thr) for which all four stereoisomers (i.e. α 'R' or 'S' and β 'R' or 'S') are commercially available. Activation of the suitably protected β-hydroxy-α-amino acids (4) via isobutyl chloroformate mixed anhydride, followed by condensation with diazomethane, yields the diazomethylketone intermediates (5). Treatment of diazomethylketone intermediates (5) with lithium chloride in aqueous acetic acid provides the protected (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide (6). Introduction of simple R¹ substituents may be achieved by condensation of activated (4) with alternatives to diazomethane such as diazoethane (R¹ = CH₃), or 1-phenyloxydiazoethane (R¹ = CH₂OPh).

The protected building blocks (synthesis exemplified by the (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide (6) detailed in Scheme 1) may be utilised in a solid phase synthesis of inhibitor molecules (steps (b) to (e)). Step (b), the solid phase linkage of an aldehyde or ketone, has previously been described by a variety of methods (e.g. see (a) James, I. W., 1999, (b) Lee, A., Huang, L., Ellman, J. A., *J. Am. Chem. Soc.*, 121(43), 9907-9914, 1999, (c) Murphy, A. M., *et al*, *J. Am. Chem. Soc.*, 114, 3156-3157, 1992). A suitable method amenable to the reversible linkage of an alkyl ketone functionality such as (6) is through a combination of the previously described chemistries. The semicarbazide, 4-[(hydrazinocarbonyl)amino] methylcyclohexane carboxylic acid.trifluoroacetate (7) (Murphy, A. M., *et al*, *J. Am. Chem. Soc.*, 114, 3156-3157, 1992), may be utilised as illustrated in Scheme 2, exemplified by linkage of the (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide (6).

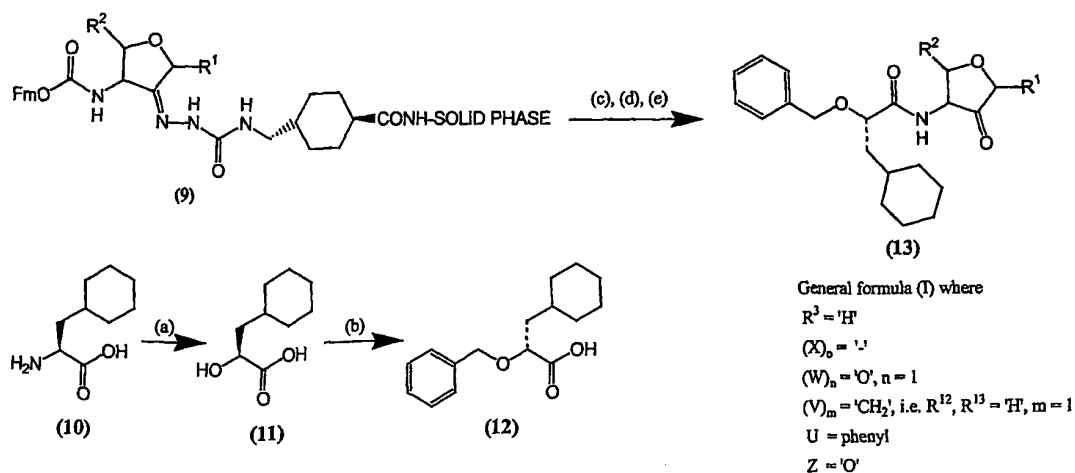


Scheme 2. (a) (6) in 90% EtOH / H₂O / 1.5eq NaOAc / 4-[[[(hydrazinocarbonyl)amino]methyl]cyclohexane carboxylic acid.trifluoroacetate (7), 2hr reflux. (b) 3 eq construct (8) / HBTU / HOBT / NMM, NH₂-SOLID PHASE, DMF, RT, o/n. (c) 20% piperidine / DMF, 30mins. (d) Range of chemistries to introduce U-V-W-X-Y (e) TFA / H₂O (95:5, v/v), RT, 2hr.

Construct (8) is prepared through reaction of the linker molecule (7) and the (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide (6) by reflux in aqueous ethanol / sodium acetate. Standard solid phase techniques (e.g. see Atherton, E. and Sheppard, R. C., 1989) are used to anchor the construct to an amino-functionalised solid phase through the free carboxylic acid functionality of (8), providing the loaded construct (9).

Loaded construct (9) may be reacted with a wide range of carboxylic acids available commercially or in the literature, to introduce the left-hand portion 'U-V-W-X-Y' in general formula (I). In the simplest example, the entire left hand portion of an inhibitor of general formula (I) can be prepared in solution by traditional organic chemistry methods and coupled to construct (9) on the solid phase (Schemes 3-7). For example (Scheme 3), treatment in solution of an amino acid, exemplified by (10) with sodium nitrite / H₂SO₄, provides the α -hydroxyacid, exemplified by (11) (Degerbeck, F. *et al*, *J. Chem. Soc. Perkin Trans. I*, 11-14, 1993). Treatment of α -hydroxyacid, (11) with sodium hydride in a dimethylformamide / dichloromethane mixture followed by addition of benzyl bromide, provides 2*RS*-benzyloxy-3-

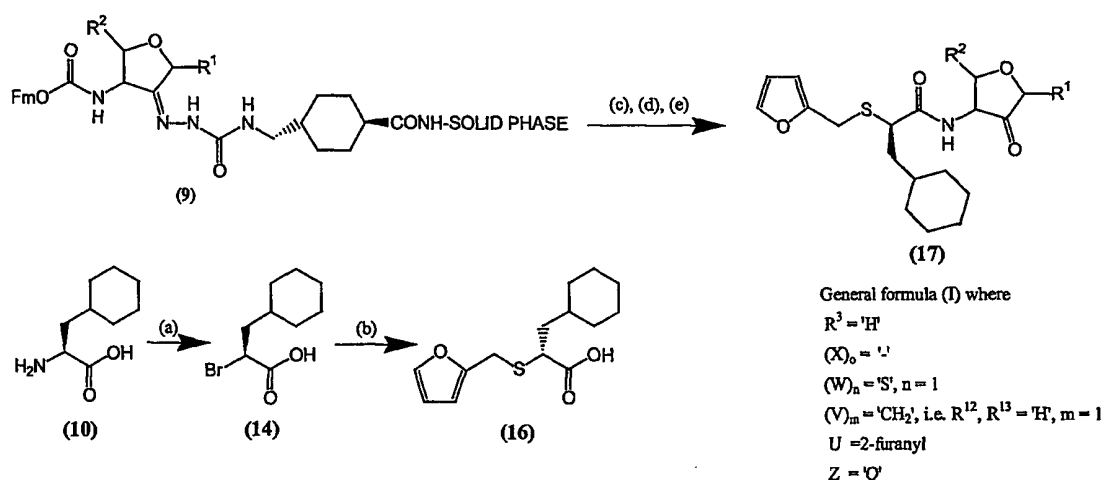
cyclohexylpropionic acid (12). Coupling of (12) to the solid phase construct (9) followed by cleavage, provides (13), an example of general formula (I) where $R^3 = 'H'$, $(X)_0 = '-'$, $(W)_n = 'O'$, $n = 1$, $(V)_m = 'CH_2'$, i.e. $R^{12}, R^{13} = 'H'$, $m = 1$ and $U =$ phenyl. To those skilled in the practices of organic synthesis, a wide variety of aminoacids such as (10) may be converted to the corresponding α -hydroxyacid such as (11) following the general conditions detailed. Additionally, benzylbromide may be replaced by any reasonable $Ar-CR^{12}R^{13}$ -halogen, providing many variations of carboxylic acid (12) following the general conditions detailed. In certain instances, it may be advantageous to temporarily protect the carboxylic acid as the methyl ester (for example compound (18), Scheme 5) prior to reaction with the alkylhalide. The ester intermediate is then simply hydrolysed to acid (12). Thus analogues of (13) exploring a wide range of $(V)_m$ and U in general formula (I) may be prepared through the general conditions detailed in Scheme 3. Since the final synthetic step involves a trifluoroacetic acid (TFA) mediated cleavage of the solid phase bound compound, compounds where the substituted ether is labile to TFA may be prepared in solution by an alternative route (see Scheme 10).



Scheme 3. (a) NaNO_2 / H_2SO_4 , $0^\circ\text{C} \rightarrow \text{RT}$, 24hr (b) 2.3eq NaH , 1:1 DMF / DCM , 1.4eq benzylbromide, o/n (c) 20% piperidine / DMF , 30mins. (d) 5eq (12) / HBTU / HOBt / NMM , DMF , RT, o/n (e) TFA / H_2O (95:5, v/v), RT, 2hr.

- 5 Alternatively, coupling of construct (9) (following removal of Fmoc) with the α -hydroxyacid (11), provides a versatile solid phase bound intermediate 'Y' substituent in general formula (I) that may be reacted with many reagents. For example, the α -hydroxyl can be reacted under Mitsunobu conditions (Hughes, D. L. *Org. React. (N.Y.)*, 42, 335-656, 1992) to give ethers (i.e. $X = '-'$, $W = 'O'$, in general formula (I)) (see Grabowska, U. *et al*, *J. Comb. Chem.*, 2(5), 475-490, 2000, for an example of Mitsunobu reaction on the solid phase). Alternatively, the α -hydroxyl can be reacted with a carbamoyl chloride to give a carbamate (i.e. $X = '-'$, $W = 'O'$, $V = 'NHC(O)'$, in general formula (I)).
- 10
- 15 Alternatively, (Scheme 4), treatment in solution of an amino acid, exemplified by (10) with sodium nitrite / H_2SO_4 / potassium bromide provides the α -bromoacid, exemplified by (14) (Souers, A. J. *et al*, *Synthesis*, 4, 583-585, 1999) with retention of configuration. Treatment of α -bromoacid (14) with an alkylthiol exemplified by furan-2-ylmethanethiol (15) in dimethylformamide / triethylamine, provides 3-cyclohexyl-2R-(furan-2-ylsulfanyl)propionic acid (16), with inversion of configuration. Coupling of (16) to the solid phase construct (9) followed by cleavage, provides (17), an example of general formula (I) where $R^3 = 'H'$, $(X)_0 = '-'$, $(W)_n = 'S'$, $n = 1$, $(V)_m = 'CH_2'$, i.e. $R^{12}, R^{13} = 'H'$, $m = 1$ and $U = 2\text{-furan-yl}$. To those
- 20

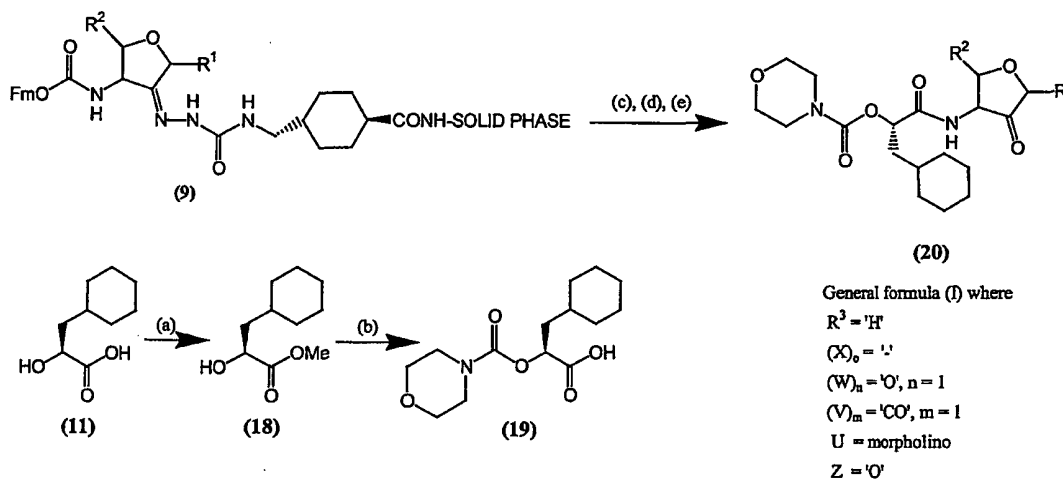
skilled in the practices of organic synthesis, a wide variety of aminoacids such as (10) may be converted to the corresponding α -bromoacid such as (14) following the general conditions detailed. Additionally, furan-2-ylmethanethiol (15) may be replaced by any reasonable $\text{Ar-CR}^{12}\text{R}^{13}\text{-SH}$, providing many variations of carboxylic acid (16) following the general conditions detailed. Thus analogues of (17) exploring a wide range of $(V)_m$ and U in general formula (I) may be prepared through the general conditions detailed in Scheme 4.



- 10 Scheme 4. (a) $\text{NaNO}_2 / \text{H}_2\text{SO}_4$, KBr $0^\circ\text{C} \rightarrow \text{RT}$, o/n (b) Alkylthiol (15) / DMF / NEt_3 , o/n (c) 20% piperidine / DMF, 30mins. (d) 5eq (16) / HBTU / HOBt / NMM, DMF, RT, o/n (e) TFA / H_2O (95:5, v/v), RT, 2hr.

Alternatively, coupling of construct (9) (following removal of Fmoc) with an α -bromoacid e.g. (14), provides a versatile intermediate 'Y' substituent in general formula (I) that may be reacted with many reagents. For example, the α -bromide can be displaced with nucleophiles e.g. alcohols, thiols, carbanions etc, to give ethers (i.e. $X = \text{'- '}$, $W = \text{'O'}$, in general formula (I)), thioethers (i.e. $X = \text{'- '}$, $W = \text{'S'}$, in general formula (I)). The thioethers may optionally be oxidised to the sulphone (see Scheme 8, i.e. $X = \text{'- '}$, $W = \text{'SO}_2\text{'}$, in general formula (I)) (see Grabowska, U. *et al*, *J. Comb. Chem.*, 2(5), 475-490, 2000, for an example of bromide displacement and thioether oxidation on the solid phase).

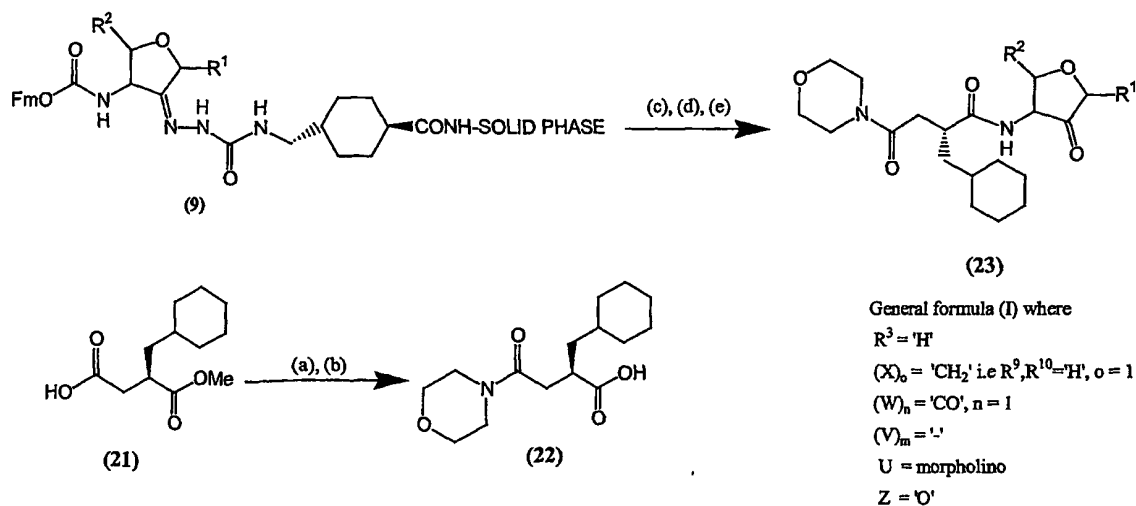
Alternatively, (Scheme 5), treatment of an α -hydroxyacid, exemplified by (11) with trimethylsilylchloride and methanol provides the methyl ester (18). Activation of the free hydroxyl to the chloroformate with phosgene in dichloromethane followed by addition of morpholine, then hydrolysis provides morpholine-4-carboxylic acid-1*S*-carboxy-2-cyclohexyl ethyl ester (19). Coupling of (19) to the solid phase construct (9) followed by cleavage, provides (20), an example of general formula (I) where $R^3 = 'H'$, $(X)_0 = '-'$, $(W)_n = 'O'$, $n = 1$, $(V)_m = 'CO'$ and $U = \text{morpholino}$. To those skilled in the practices of organic synthesis, a wide variety of α -hydroxyacid esters such as (18) could be converted to the activated chloroformate following the general conditions detailed. Additionally, morpholine may be replaced by any reasonable amine, providing many variations of carboxylic acid (19) following the general conditions detailed. Thus analogues of (20) exploring a wide range of $(V)_m$ and U in general formula (I) may be prepared through the general conditions detailed in Scheme 5.



Scheme 5. (a) Me_3SiCl , MeOH , RT, o/n.. (b) i. COCl_2 / DCM / o/n, ii. Morpholine / DCM 0°C , 2hr, iii. LiOH in H_2O / dioxan, 0°C . (c) 20% piperidine / DMF , 30mins. (d) 5eq (19) / HBTU / HOBt / NMM, DMF , RT, o/n (e) TFA / H_2O (95:5, v/v), RT, 2hr.

Alternatively, (Scheme 6), a wide range of alkylsuccinate esters exemplified by 2*R*-cyclohexylmethylsuccinic acid 1-methyl ester (21) are commercially available or readily prepared by known methods (see (a) Azam *et al*, *J. Chem. Soc. Perkin Trans.*

- 1, 621-, 1996; (b) Evans *et al*, *J. Chem. Soc. Perkin Trans. 1*, 103, 2127, 1981; (c) Oikawa *et al*, *Tet. Lett*, 37, 6169, 1996). Carboxyl activation of alkylsuccinate ester (21) followed by addition of morpholine in dimethylformamide and subsequent ester hydrolysis, provides 2*R*-cyclohexylmethyl-4-morpholin-4-yl-4-oxo-butyric acid (22).
- 5 Coupling of (22) to the solid phase construct (9) followed by cleavage, provides (23), an example of general formula (I) where $R^3 = 'H'$, $(X)_o = 'CH_2'$ i.e. $R^9, R^{10} = 'H'$, $o = 1$, $(W)_n = 'CO'$, $n = 1$, $(V)_m = '-'$ and $U = \text{morpholino}$. To those skilled in the practices of organic synthesis, a wide variety of alkylsuccinate esters such as (21) may be prepared and converted to the corresponding substituted alkylsuccinate acid (22) following the general conditions detailed. Additionally, morpholine may be replaced by any reasonable amine, providing many variations of carboxylic acid (22) following the general conditions detailed. Thus analogues of (23) exploring a wide range of $(X)_o$, $(V)_m$ and U in general formula (I) may be prepared through the general conditions detailed in Scheme 6.



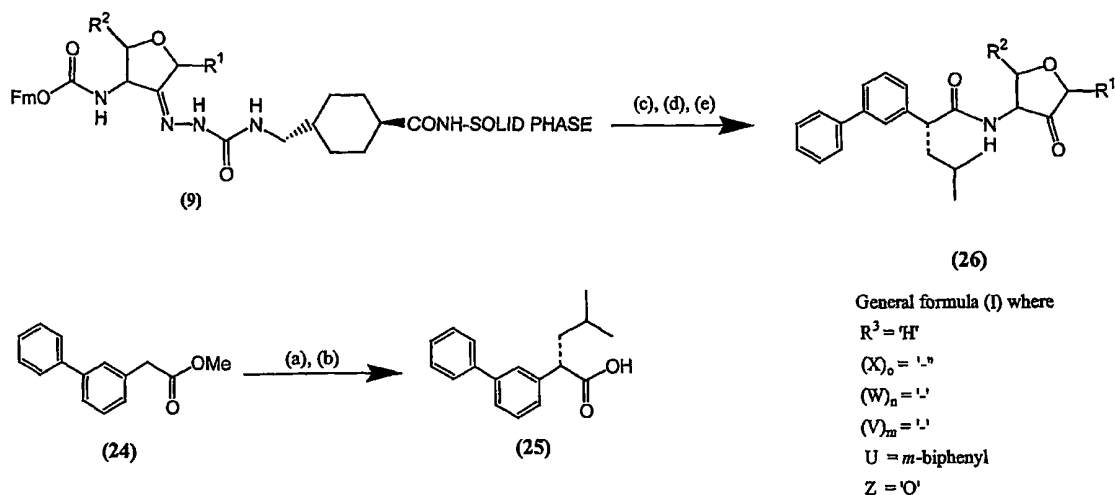
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Scheme 6. (a) i.EDC / 1-hydroxybenzotriazole /DMF, 0°C, 30mins. ii. Morpholine, RT, o/n (b) LiOH in H₂O / dioxan, 0°C (c) 20% piperidine / DMF, 30mins. (d) 5eq (22) / HBTU / HOBt / NMM, DMF, RT, o/n (e) TFA / H₂O (95:5, v/v), RT, 2hr.

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Alternatively, (Scheme 7), a wide range of biarylalkylacetic acids, exemplified by 2*RS*-biphenyl-3-yl-4-methylpentanoic acid (25) are readily available by known methods (see (a) DesJarlais, R. L. *et al*, *J. Am. Chem. Soc.*, 120, 9114-9115, 1998; (b)

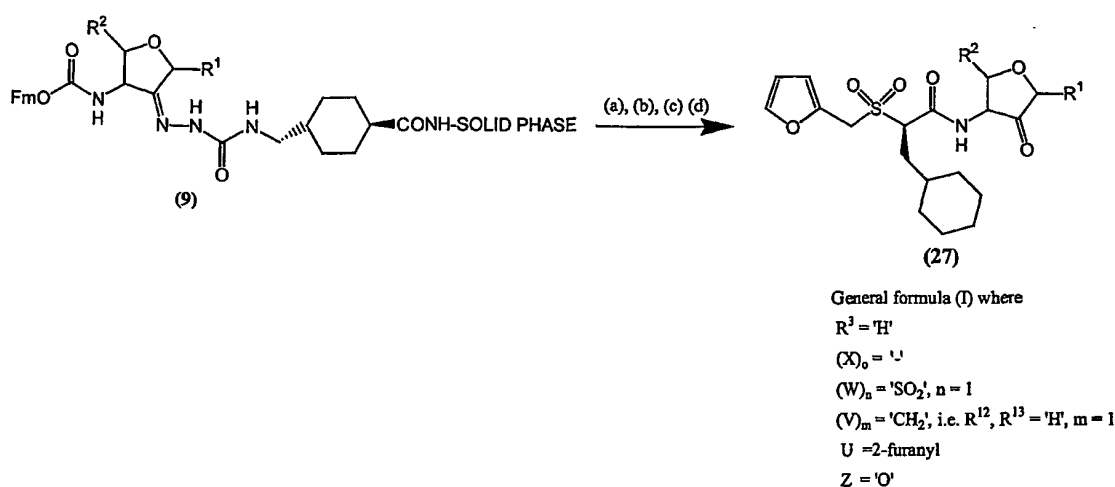
Oballa, R. M. *et al*, WO 0149288). Coupling of biarylalkylacetic acid (25) to the solid phase construct (9) followed by cleavage, provides (26), an example of general formula (I) where $R^3 = 'H'$, $(X)_o = '-'$, $(W)_n = '-'$, $(V)_m = '-'$ and $U = m$ -biphenyl. To those skilled in the practices of organic synthesis, a wide variety of biarylalkylacetic acids such as (25) may be prepared by alkylation of the α -anion of the free acid analogue of (24), which in turn is prepared by Suzuki coupling of phenylboronic acid and 3-bromophenylacetic acid methyl ester. Phenylboronic acid may be replaced by a wide range of arylboronic acids in the Suzuki coupling, providing many variations of carboxylic acid (25) following the general conditions detailed. Thus analogues of (26) exploring a wide range of group 'U' in general formula (I) may be prepared through the general conditions detailed in Scheme 7.



Scheme 7. (a) LiOH in H_2O / dioxan, $0^\circ C$ (b) i.LDA, THF, 2-methylpropenylbromide. ii. Pd/C, EtOH, H_2 (c) 20% piperidine / DMF, 30mins. (d) 5eq (25) / HBTU / HOBt / NMM, DMF, RT, o/n (e) TFA / H_2O (95:5, v/v), RT, 2hr.

Many other possibilities for solid phase organic chemistry (e.g. see Brown, R. D. *J. Chem. Soc., Perkin Trans.1*, 19, 3293-3320, 1998, for a review of recent SPOC publications) can be used to derivatise construct (9) towards compounds of general formula (I). For example, the left-hand portion 'U-V-W-X-Y' in general formula (I) can be partially constructed in solution, coupled to construct (9) and further modified on the solid phase (Scheme 8). For instance, a simple extension of Scheme 4 is

through the oxidation of the intermediate solid phase bound species, with *m*-chloroperbenzoic acid in dichloromethane prior to cleavage, to give the sulphone analogue (27). As described in Scheme 4, many variations of carboxylic acid (16) may be prepared following the general conditions detailed. Thus analogues of (27) exploring a wide range of (V)_m and U in general formula (I) may be prepared through the general conditions detailed in Schemes 4 and 8.



Scheme 8. (a) 20% piperidine / DMF, 30mins. (b) 5eq (16) / HBTU / HOBt / NMM, DMF, RT, o/n
 (c) 5eq *m*-chloroperbenzoic acid / DCM, RT, 5hr. (d) TFA / H₂O (95:5, v/v), RT, 2hr.

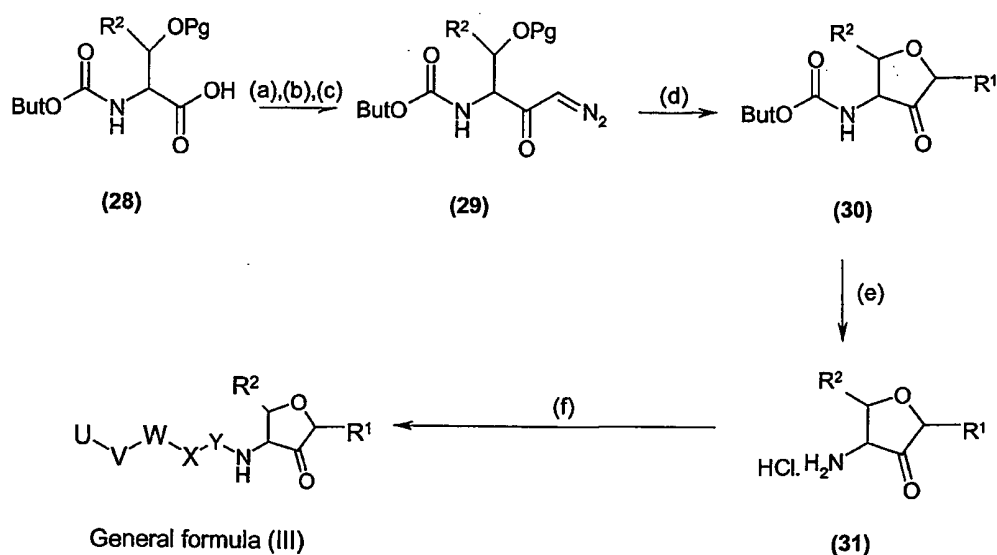
Compounds of general formula (I) can be finally released from the solid phase by treatment with trifluoroacetic acid / water, followed by evaporation, lyophilisation and standard analytical characterisation.

A second strategy for the synthesis of compounds of general formula (I) comprises:-

(f) Preparation of an appropriately functionalised and protected (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide or a (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amide or a (2-alkyl-5-oxocyclopentyl)amide building block in solution. Preferred protecting groups for solution phase chemistry are the *N*α-*tert*-butoxycarbonyl group and the *N*α-benzyloxycarbonyl group.

- (g) Standard organic chemistry methods for the conversion of building block (f) towards compounds of general formula (I).

In the simplest example, the entire left hand portion of an inhibitor of general formula (I) can be prepared in solution by traditional organic chemistry methods and coupled to building block (f) (see Scheme 9 exemplified by preparation and use of the (2-alkyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid tert-butyl ester (30)).



Scheme 9. (a) $t\text{BuOCOCN}$, NMM, DCM, -15°C , 10mins, under argon. (b) Diazomethane in diethyl ether, -15°C to RT over 1hr. (c) Acetic acid (d) LiCl (10eq) in 80%aq acetic acid, 5°C to RT over 1hr. (e) 4M HCl in dioxan, 0°C , 2hrs. (f) Pre-prepared $\text{U}-\text{V}-\text{W}-\text{X}-\text{Y}-\text{COOH}$ / HBTU / HOBT / NMM, DMF, RT, o/n.

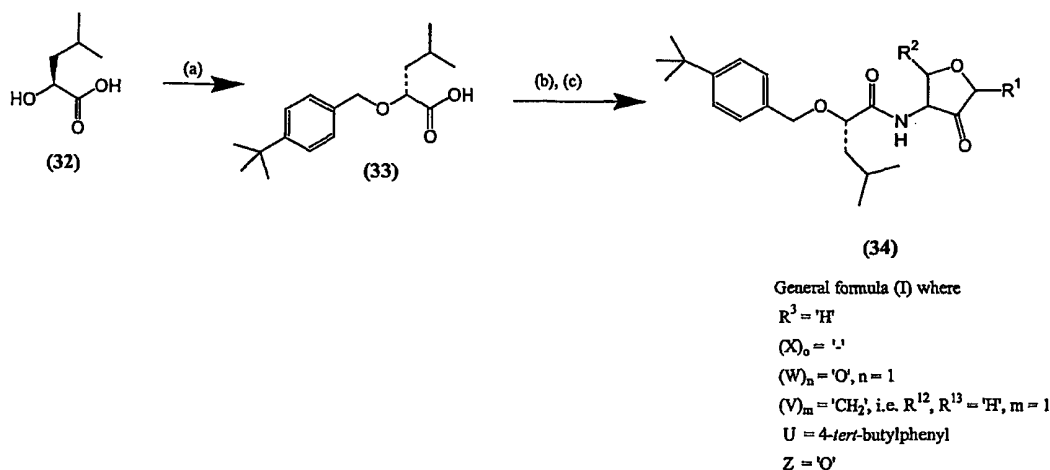
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The general strategy detailed in Scheme 9 is particularly useful when the compound of general formula (I) contains a substituent that is labile to trifluoroacetic acid, this being the final reagent used in each of the solid phase Schemes 3-8. For example (Scheme 10), treatment in solution of α -hydroxyacid (32) with sodium hydride in a dimethylformamide / dichloromethane mixture followed by addition of 4-*tert*-butylbenzyl bromide, provides 2*RS*-(4-*tert*-butylbenzyloxy)-4-methylpentanoic acid (33). Coupling of (33) to hydrochloride salt (31), provides (34), an example of general formula (I) where $\text{R}^3 = \text{'H'}$, $(\text{X})_0 = \text{'-'}'$, $(\text{W})_n = \text{'O'}$, $n = 1$, $(\text{V})_m = \text{'CH}_2'$, i.e.

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$R^{12}, R^{13} = 'H'$, $m = 1$ and $U = 4\text{-}tert\text{-butylphenyl}$. To those skilled in the practices of organic synthesis, 4-*tert*-butylbenzyl bromide may be replaced by any reasonable $Ar-CR^{12}R^{13}$ -halogen, providing many variations of carboxylic acid (33) under the conditions shown. Thus analogues of (34) exploring a wide range of $(V)_m$ and U in

5 general formula (I) may be prepared through the conditions detailed in Scheme 10.



Scheme 10. (a) 2.2eq NaH, 1:1 DMF / DCM, 1.25eq 4-*tert*-benzylbromide, 2hr (b) $tBuOCOC1$, NMM, DCM, $-15^\circ C$, 1hr, under nitrogen. (c) 1eq (31), NMM, RT, o/n .

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A third strategy for the synthesis of compounds of general formula (I) where the addition of U-V-W-X-Y to the protected (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide or a (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amide or a (2-alkyl-5-oxocyclopentyl)amide building block involves multistep organic reactions comprises:-

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(h) Preparation of an appropriately functionalised and protected (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide or a (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amide or a (2-alkyl-5-oxocyclopentyl)amide building block in solution. Preferred protecting groups for solution phase chemistry are the $N\alpha$ -*tert*-butoxycarbonyl group and the $N\alpha$ -benzyloxycarbonyl group.

20

(i) Protection of the ketone functionality of the (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide or a (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amide or a (2-alkyl-5-oxocyclopentyl)amide building block e.g. as a dimethylacetal. Alternatively, the

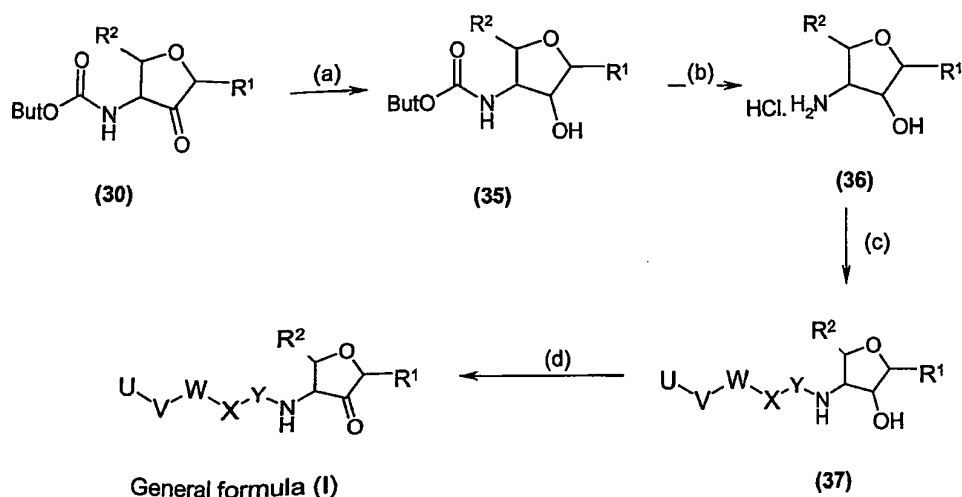
ketone may be reduced to the achiral secondary alcohols and re-oxidised as the final synthetic step.

- (j) Standard organic chemistry methods for the conversion of building block (i) towards compounds of general formula (I).

5

Intermediates may be prepared in solution, followed by coupling to building block (i) and further derivitisation towards compounds of general formula (I) (see Scheme 11 exemplified by preparation and use of the (4-Hydroxy-2-alkyl-tetrahydrofuran-3-yl) carbamic acid tert-butyl ester (35)).

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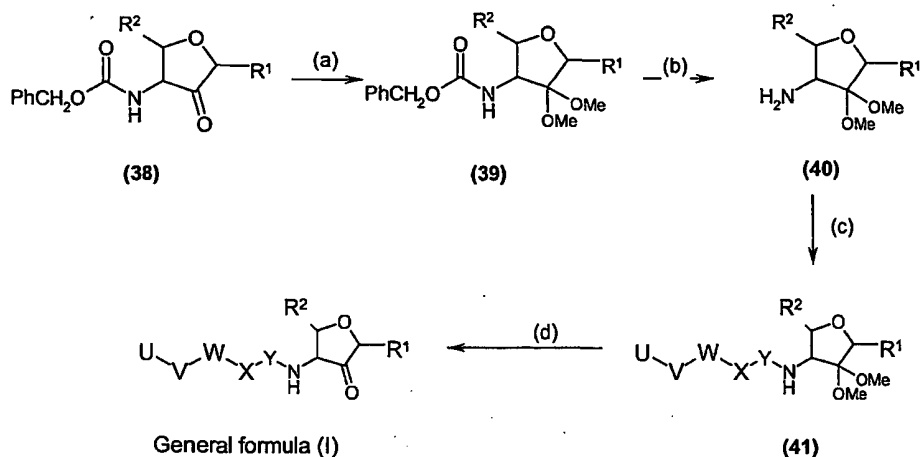


Scheme 11. (a) Reduction, e.g. NaBH_4 (b) 4M HCl in dioxan, 0°C , 2hrs. (c) Stepwise reaction with intermediates of Y, then X, then W etc., to stepwise construct compounds (37). (d) Oxidation, e.g. Dess-Martin periodane, CH_2Cl_2 .

Alternatively, depending upon the types of chemistry used to construct the left hand side U-V-W-X-Y of compounds of general formula (I), the ketone may require protection e.g. as the dimethyl acetal. Such a method is detailed and exemplified in Scheme 12 by the preparation and use of (4,4-Dimethoxy-2-alkyl-tetrahydrofuran-3-yl)-carbamic acid benzyl ester (38).

20

-44-



Scheme 12. (a) Triethylorthoformate / pTSA / MeOH. (b) H₂, Pd-C. (c) Stepwise reaction with intermediates of Y, then X, then W etc., to stepwise construct compounds (41). (d) Trifluoroacetic acid / CH₂Cl₂ / H₂O.

5

The invention extends to novel intermediates as described above, and to processes for preparing compounds of general formula (I) from each of its immediate precursors. In turn, processes for preparing intermediates from their immediate precursors also form part of the invention.

10

Compounds of general formula (I) are useful both as laboratory tools and as therapeutic agents. In the laboratory certain compounds of the invention are useful in establishing whether a known or newly discovered cysteine protease contributes a critical or at least significant biochemical function during the establishment or progression of a disease state, a process commonly referred to as 'target validation'.

15

According to a second aspect of the invention, there is provided a method of validating a known or putative cysteine protease inhibitor as a therapeutic target, the method comprising:

20

- (a) assessing the *in vitro* binding of a compound as described above to an isolated known or putative cysteine protease, providing a measure of potency; and optionally, one or more of the steps of:

- (b) assessing the binding of the compound to closely related homologous proteases of the target and general house-keeping proteases (e.g. trypsin) to provides a measure of selectivity;
- 5 (c) monitoring a cell-based functional marker of a particular cysteine protease activity, in the presence of the compound; and
- (d) monitoring an animal model-based functional marker of a particular cysteine protease activity in the presence of the compound.

10

The invention therefore provides a method of validating a known or putative cysteine protease inhibitor as a therapeutic target. Differing approaches and levels of complexity are appropriate to the effective inhibition and 'validation' of a particular target. In the first instance, the method comprises assessing the *in vitro* binding of a

15 compound of general formula (I) to an isolated known or putative cysteine protease, providing a measure of 'potency'. An additional assessment of the binding of a compound of general formula (I) to closely related homologous proteases of the target and general house-keeping proteases (e.g. trypsin) provides a measure of 'selectivity'. A second level of complexity may be assessed by monitoring a cell-

20 based functional marker of a particular cysteine protease activity, in the presence of a compound of general formula (I). For example, a 'human osteoclast resorption assay' has been utilised as a cell-based secondary *in vitro* testing system for monitoring the activity of cathepsin K and the biochemical effect of protease inhibitors (e.g. see WO-A-9850533). An 'MHC-II processing – T-cell activation assay' has been utilised

25 as a cell-based secondary *in vitro* testing system for monitoring the activity of cathepsin S and the biochemical effect of protease inhibitors (Shi, G-P., *et al*, *Immunity*, 10, 197-206, 1999). When investigating viral or bacterial infections such a marker could simply be a functional assessment of viral (e.g. count of mRNA copies) or bacterial loading and assessing the biochemical effect of protease inhibitors. A

30 third level of complexity may be assessed by monitoring an animal model-based functional marker of a particular cysteine protease activity, in the presence of a compound of general formula (I). For example, murine models of *Leishmania*

infection, *P. vinckei* infection, malaria (inhibition of falcipain) and *T. cruzi* infection (cruzipain), indicate that inhibition of cysteine proteases that play a key role in pathogen propagation is effective in arresting disease symptoms, 'validating' said targets.

5

The invention therefore extends to the use of a compound of general formula (I) in the validation of a known or putative cysteine protease inhibitor as a therapeutic target.

- 10 Compounds of general formula (I) are useful for the *in vivo* treatment or prevention of diseases in which participation of a cysteine protease is implicated.

According to a third aspect of the invention, there is provided a compound of general formula (I) for use in medicine, especially for preventing or treating diseases in
15 which the disease pathology may be modified by inhibiting a cysteine protease.

According to a fourth aspect of the invention, there is provided the use of a compound of general formula (I) in the preparation of a medicament for preventing or treating diseases in which the disease pathology may be modified by inhibiting a
20 cysteine protease.

Certain cysteine proteases function in the normal physiological process of protein degradation in animals, including humans, *e.g.* in the degradation of connective tissue. However, elevated levels of these enzymes in the body can result in
25 pathological conditions leading to disease. Thus, cysteine proteases have been implicated in various disease states, including but not limited to, infections by *Pneumocystis carinii*, *Trypanoma cruzi*, *Trypanoma brucei brucei* and *Crithidia fusciculata*; as well as in osteoporosis, autoimmunity, schistosomiasis, malaria, tumour metastasis, metachromatic leukodystrophy, muscular dystrophy, amyotrophy,
30 and the like. See WO-A-9404172 and EP-A-0603873 and references cited in both of them. Additionally, a secreted bacterial cysteine protease from *S. Aureus* called

staphylopain has been implicated as a bacterial virulence factor (Potempa, J., *et al. J. Biol. Chem.*, 262(6), 2664-2667, 1998).

The invention is useful in the prevention and/or treatment of each of the disease states mentioned or implied above. The present invention also is useful in a methods of treatment or prevention of diseases caused by pathological levels of cysteine proteases, particularly cysteine proteases of the papain superfamily, which methods comprise administering to an animal, particularly a mammal, most particularly a human, in need thereof a compound of the present invention. The present invention particularly provides methods for treating diseases in which cysteine proteases are implicated, including infections by *Pneumocystis carinii*, *Trypanosoma cruzi*, *Trypanosoma brucei*, *Leishmania mexicana*, *Clostridium histolyticum*, *Staphylococcus aureus*, foot-and-mouth disease virus and *Crithidia fusciculata*; as well as in osteoporosis, autoimmunity, schistosomiasis, malaria, tumour metastasis, metachromatic leukodystrophy, muscular dystrophy and amyotrophy.

Inhibitors of cruzipain, particularly cruzipain-specific compounds, are useful for the treatment of Chagas' disease.

In accordance with this invention, an effective amount of a compound of general formula (I) may be administered to inhibit the protease implicated with a particular condition or disease. Of course, this dosage amount will further be modified according to the type of administration of the compound. For example, to achieve an "effective amount" for acute therapy, parenteral administration of a compound of general formula (I) is preferred. An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although an intramuscular bolus injection is also useful. Typically, the parenteral dose will be about 0.01 to about 100 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the concentration of drug in the plasma at a concentration effective to inhibit a cysteine protease. The compounds may be administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 400 mg/kg/day. The precise amount of an inventive compound which is

therapeutically effective, and the route by which such compound is best administered, is readily determined by one of ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect. Prodrugs of compounds of the present invention may be prepared by any suitable method. For those compounds in which the prodrug moiety is a ketone functionality, specifically ketals and/or hemiacetals, the conversion may be effected in accordance with conventional methods.

The compounds of this invention may also be administered orally to the patient, in a manner such that the concentration of drug is sufficient to inhibit bone resorption or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg.

No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention. The compounds of this invention, which may have good bioavailability, may be tested in one of several biological assays to determine the concentration of a compound which is required to have a given pharmacological effect.

According to a fifth aspect of the invention, there is provided a pharmaceutical or veterinary composition comprising one or more compounds of general formula (I) and a pharmaceutically or veterinarily acceptable carrier. Other active materials may also be present, as may be considered appropriate or advisable for the disease or condition being treated or prevented.

The carrier, or, if more than one be present, each of the carriers, must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient.

The formulations include those suitable for rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration, but preferably the formulation is an orally administered formulation. The formulations may conveniently be presented in unit dosage form, e.g. tablets and sustained release capsules, and may be prepared by any methods well known in the art of pharmacy.

Such methods include the step of bringing into association the above defined active agent with the carrier. In general, the formulations are prepared by uniformly and intimately bringing into association the active agent with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product. The invention extends to methods for preparing a pharmaceutical composition comprising bringing a compound of general formula (I) in conjunction or association with a pharmaceutically or veterinarily acceptable carrier or vehicle.

Formulations for oral administration in the present invention may be presented as: discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion; or as a bolus etc.

For compositions for oral administration (e.g. tablets and capsules), the term "acceptable carrier" includes vehicles such as common excipients e.g. binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and lubricants such as magnesium stearate, sodium stearate and other metallic stearates, glycerol stearate stearic acid, silicone fluid, talc waxes, oils and colloidal silica. Flavouring agents such as peppermint, oil of wintergreen, cherry flavouring and the like can also be used. It may be desirable to add a colouring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art.

- A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active agent in a free flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agent.
- 10 Other formulations suitable for oral administration include lozenges comprising the active agent in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active agent in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active agent in a suitable liquid carrier.
- 15 Parenteral formulations will generally be sterile.

According to a sixth aspect of the invention, there is provided a process for the preparation of a pharmaceutical or veterinary composition as described above, the process comprising bringing the active compound(s) into association with the carrier, for example by admixture.

Preferred features for each aspect of the invention are as for each other aspect *mutatis mutandis*.

- 25 The invention will now be illustrated with the following examples:

Solution Phase Chemistry – General Methods

All solvents were purchased from ROMIL Ltd (Waterbeach, Cambridge, UK) at SpS or Hi-Dry grade unless otherwise stated. General peptide synthesis reagents were obtained from Chem-Impex Intl. Inc. (Wood Dale IL 60191. USA). Thin layer chromatography (TLC) was performed on pre-coated plates (Merck aluminium

5 sheets silica 60 F254, part no. 5554). Visualisation of compounds was achieved under ultraviolet light (254nm) or by using an appropriate staining reagent. Flash column purification was performed on silica gel 60 (Merck 9385). All analytical HPLC were obtained on Phenomenex Jupiter C₄, 5 μ , 300A, 250 x 4.6mm, using
10 mixtures of solvent A = 0.1%aq trifluoroacetic acid (TFA) and solvent B = 90% acetonitrile / 10% solvent A on automated Agilent systems with 215 and / or 254nm UV detection. Unless otherwise stated a gradient of 10 – 90% B in A over 25 minutes at 1.5mL / min was performed for full analytical HPLC analysis. HPLC-MS analysis was performed on an Agilent 1100 series LC/MSD, using automated Agilent
15 HPLC systems, with a gradient of 10 – 90% B in A over 10 minutes on Phenomenex Columbus C₈, 5 μ , 300A, 50 x 2.0mm at 0.4mL / min. Nuclear magnetic resonance (NMR) were obtained on a Bruker DPX400 (400MHz ¹H frequency; QXI probe) in the solvents and temperature indicated. Chemical shifts are expressed in parts per million (δ) and are referenced to residual signals of the solvent. Coupling constants
20 (*J*) are expressed in Hz.

Example inhibitors (1-47) were prepared through a combination of solution chemistry and solid phase Fmoc-based chemistries (see 'Solid Phase Peptide Synthesis', Atherton, E. and Sheppard, R. C., IRL Press Ltd, Oxford, UK, 1989, for a
20 general description) (Schemes 1-12).

Solid Phase Chemistry – General Methods

25 An appropriately protected and functionalised building block was prepared in solution (e.g. general compound (6), Scheme 1), then reversibly attached to the solid phase through an appropriate linker. Rounds of coupling / deprotection / chemical modification e.g. oxidation, were then performed until the full length desired molecule was complete (Scheme 2). Example inhibitors (1-47) were then released (cleaved) from the solid phase, analysed, purified and assayed for inhibition verses a
30 range of proteases.

Generally, multipins (polyamide 1.2 \rightarrow 10 μ mole loadings, see www.mimotopes.com) were used for the solid phase synthesis, although any suitable solid phase surface could be chosen. In general, the 1.2 μ mole gears were used to provide small scale crude examples for preliminary screening, whilst the 10 μ mole crowns were used for scale-up synthesis and purification of preferred examples. Standard coupling and Fmoc deprotection methods were employed (see Grabowska, U. *et al*, *J. Comb. Chem.* 2(5), 475-490, 2000. for a thorough description of solid phase multipin methodologies).

10 Preparation of Initial Assembly

Building Block-linker constructs (e.g.(8), typically 100mg to 2g) were carboxyl activated with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro phosphate (HBTU, 1 mole equivalent), 1-hydroxybenzotriazole.hydrate (HOBT, 1 mole equivalent) and N-methylmorpholine (NMM, 2 mole equivalents) in dimethylformamide (DMF, typically 1 to 10mL) for 5 minutes. Amino functionalised DA/MDA crowns or HEMA gears (10 μ mole per crown / 1.2 μ mole per gear, 0.33 mole equivalent of total surface amino functionalisation compared to activated construct) were added, followed by additional DMF to cover the solid phase surface. The loading reaction was left overnight. Following overnight loading, crowns / gears were taken through standard cycles washing, Fmoc deprotection and loading quantification (see Grabowska, U. *et al*) to provide loaded Building Block-linker constructs (e.g.(9)). Analysis indicated virtually quantitative loading in all examples.

25

Coupling Cycles

The coupling of standard Fmoc-aminoacids and novel carboxylic acids (e.g. (12) Scheme 3, (16) Scheme 4, (19) Scheme 5, (22) Scheme 6, (25) Scheme 7) (10 or 20 mole equivalent) were performed via carboxyl activated with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 10 or 20mole equivalent), 1-hydroxybenzotriazole.hydrate (HOBT, 10 or 20mole equivalent) and

N-methylmorpholine (NMM, 20 or 40mole equivalents) in dimethylformamide, with pre-activation for 5 minutes. Activated species were dispensed to the appropriate wells of a polypropylene 96-well plate (Beckman, 1mL wells, 500µL solution per well for crowns or 250µL solution per well for gears) in a pattern required for synthesis. Loaded free amino Building Block-linker constructs (e.g.(9)) were added and the coupling reaction left overnight. Following overnight coupling, crowns / gears were taken through standard cycles washing and Fmoc deprotection (see Grabowska, U. *et al*). Identical activation and coupling conditions were used for the coupling of a range of carboxylic acids (R-COOH). Alternatively, chloroformates e.g. morpholine-4-carbonylchloride (10 mole equivalent), were coupled in DMF with the addition of NMM (10mole equivalents).

Acidolytic Cleavage Cycle

A mixture of 95% TFA / 5% water was pre-dispensed into two polystyrene 96-well plates (Beckman, 1mL wells, 600µL solution per well for crowns or 300µL solution per well for gears) in a pattern corresponding to that of the synthesis. The completed multipin assembly was added to the first plate (mother plate), the block covered in tin foil and cleaved for 2 hours. The cleaved multipin assembly was then removed from the first plate and added to the second plate (washing plate) for 15 minutes. The spent multipin assembly was then discarded and the mother / washing plates evaporated on a HT-4 GeneVac plate evaporator.

Analysis and Purification of Cleaved Examples

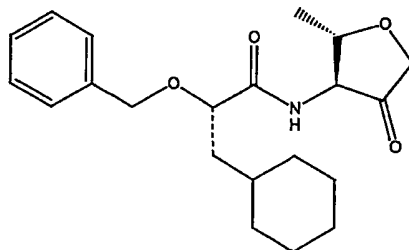
25

(a) Ex 1.2µmole Gears. 100µL dimethylsulphoxide (DMSO) was added to each post cleaved and dried washing plate well, thoroughly mixed, transferred to the corresponding post cleaved and dried mother plate well and again thoroughly mixed. 10µL of this DMSO solution was diluted to 100µL with a 90% acetonitrile / 10% 0.1%aq TFA mixture. 20µL aliquots were analysed by HPLC-MS and full analytical HPLC. In each case the crude example molecules gave the expected $[M + H]^+$ ion and an HPLC peak at > 80% (by

215nm UV analysis). This provided an approximately 10mM DMSO stock solution of good quality crude examples for preliminary protease inhibitory screening.

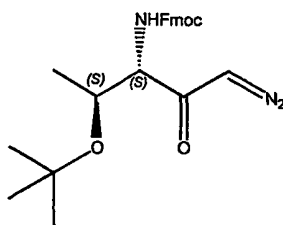
- 5 (b) Ex 10μmole Crowns. 500μL of a 90% acetonitrile / 10% 0.1%aq TFA mixture was added to each washing plate well, thoroughly mixed, transferred to the corresponding mother plate well and again thoroughly mixed. 5μL of this solution was diluted to 100μL with a 90% acetonitrile / 10% 0.1%aq TFA mixture. 20μL aliquots were analysed by HPLC-MS and full analytical HPLC. In each case the crude example molecules gave the expected $[M + H]^+$ ion and an HPLC peak at > 80% (by 215nm UV analysis). The polystyrene blocks containing crude examples were then lyophilised.
- 10 (c) Individual examples (ex (b)) were re-dissolved in a 1 : 1 mixture of 0.1% aq TFA / acetonitrile (1mL) and purified by semi-preparative HPLC (Phenomenex Jupiter C₄, 5μ, 300A, 250 x 10mm, a 25-90% B in A gradient over 25mins, 4.0mL/min, 215nm UV detection). Fractions were lyophilised into pre-tarred glass sample vials to provide purified examples (typically 2 to 4mg, 40 to 80% yield).
- 15 (d) Purified examples were dissolved in an appropriate volume of DMSO to provide a 10mM stock solution, for accurate protease inhibitory screening.
- 20

EXAMPLE 1. (2*S*, 3*S*) 2-Benzoyloxy-3-cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydro furan-3-yl)-propionamide



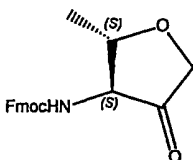
Following the general details from Scheme 1, the required bicycle building block (2*S*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9H-fluoren-9-ylmethyl ester (6a) was prepared as follows:

- 5 (1) Preparation of (1*S*, 1'*S*) [3-diazo-1-(1-*tert*-butoxyethyl)-2-oxo-propyl]-carbamic acid 9H-fluoren-9-ylmethyl ester.



- A solution of *iso*-butyl chloroformate (1.05 g, 7.7 mmol) in dichloromethane (14 ml) and a solution of 4-methylmorpholine (1.42 g, 14 mmol) in dichloromethane (14 ml) were simultaneously added to a stirred suspension of Fmoc-*O*-(*tert*-butyl ether)-L-*allo*-threonine (2.78g, 7mmol) in dichloromethane (70 ml) at -15 °C over 10 minutes under an atmosphere of nitrogen. Ethereal diazomethane [generated from diazald (7.14 g, ~21mmol) addition in diethyl ether (115 ml) to sodium hydroxide (8.022 g) in water (11 ml)/ethanol (31 ml) at 60°C] was then cautiously added and the resulting yellow solution was stirred at room temperature for 90 minutes. Acetic acid (~3 ml) was cautiously added (until effervescence had ceased) then the mixture was diluted with *tert*-butyl methyl ether (70 ml). The ethereal layer was washed with water (3 x 70 ml), dried (Na₂SO₄) and the solvent removed *in vacuo* to leave (1*S*, 1'*S*) [3-diazo-1-(1-*tert*-butoxyethyl)-2-oxo-propyl]-carbamic acid 9H-fluoren-9-ylmethyl ester as a yellow oil (5.47 g) which was used without further purification.

- (2) Cyclisation to (2*S*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9H-fluoren-9-ylmethyl ester.



A solution of lithium chloride (2.97 g, 70mmol) in 80% acetic acid in water (105 ml) was added to (1*S*, 1'*S*) [3-diazo-1-(1-*tert*-butoxyethyl)-2-oxo-propyl]carbamic acid 9H-fluoren-9-ylmethyl ester (5.7 g, ~7mmol). The yellow oily suspension was stirred for 2 hours whereupon a pale yellow solution formed accompanied by the evolution
5 of a gas. The solvents were removed *in vacuo* then the residue was dissolved in ethyl acetate (42 ml), washed with 10% aqueous sodium carbonate solution (2 x 42 ml) and saturated aqueous sodium chloride solution (42 ml). The combined aqueous layers were extracted with ethyl acetate (2 x 42 ml) then the combined ethyl acetate layers were dried (Na₂SO₄) and the solvents removed *in vacuo* to leave a yellow gum
10 (4.28 g). The yellow gum was purified by chromatography over silica gel eluting with a gradient of n-heptane : ethyl acetate 9:1 → 7:3. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (2*S*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9H-fluoren-9-ylmethyl ester as a white crystalline solid (1.58 g, 67% from starting acid). TLC (single UV spot, R_f = 0.27, n-
15 heptane : ethyl acetate 2:1), analytical HPLC peak Rt = 17.92mins, HPLC-MS (single UV peak with Rt = 8.47mins, 360.0 [M + Na]⁺, 361.0 [M + H + Na]⁺).

¹H (CDCl₃ at 298K); 1.28-1.50 (3H, CH₃CHO, brs), 3.60-3.75 (1H, CH₃CHO m), 3.78-4.00 (2H, Fmoc CH₂, m), 4.04-4.24 (2H, Fmoc H-9 and NHCHCO, m), 4.26-
20 4.50 (2H, OCH₂CO, dd), 4.92-5.14 (1H, NH, brs), 7.17-7.36 (4H, ArH, Fmoc H-2, H-3, H-6 and H-7), 7.40-7.56 (2H, ArH, Fmoc H-1 and H-8), 7.60-7.75 (2H, ArH, Fmoc H-4 and H-5).

¹³C (CDCl₃ at 298K); 19.44 (u, CH₃CHO), 47.52 (u, Fmoc C-9), 63.09 (u, NHCHCO), 67.65 (d, Fmoc CH₂), 71.20 (d, COCH₂), 77.82 (u, CH₃CHO), 120.43
25 (u, Fmoc C-4 and C-5), 125.38 (u, Fmoc C-1 and C-8), 127.51 (u, Fmoc C-2 and C-7), 128.19 (u, Fmoc C-3 and C-6), 141.75 (q, Fmoc C-4' and C-5'), 143.98/144.03 (q, Fmoc C-1' and C-8'), 156.42 (q, OCON), 212.18 (q, COCH₂).

30 Following the general details from Scheme 2, the required bicycle building (2*S*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9H-fluoren-9-ylmethyl ester (6a) was converted to building block-linker construct (8a) as follows:

(2*S*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9H-fluoren-9-ylmethyl ester (**6a**) (354mg, 1.05mmol, 1 eq) was dissolved in a mixture of ethanol (9.6 mL) and water (1.4 mL) containing sodium acetate.trihydrate (218mg, 1.6 mmol, 1.5 eq). 4-[[[(hydrazinocarbonyl)amino] methyl]cyclohexanecarboxylic acid. trifluoroacetate (346mg, 1.05mmol, 1 eq, Murphy, A. M. *et al*, *J. Am. Chem. Soc.*, 114, 3156-3157, 1992) was added and the mixture refluxed for 2hr. Chloroform (100mL) was added and the organics washed with dilute aqueous hydrochloric acid (0.1 M, 1 x 100 mL). The acidic layer was backwashed with chloroform (3 x 100mL) and the combined organics washed with brine (100 mL), dried (Na₂SO₄) and evaporated under reduced pressure to afford linker construct (**8a**) as a white solid (630mg). Analytical HPLC indicated one main peak at *R*_t = 17.17 min, HPLC-MS (main UV peak with *R*_t = 7.91 min, 535.3 [M + H]⁺. Crude (**8a**) was used directly for construct loading.

Following the general details from Scheme 2, the required building block-linker construct (**8a**) was attached to the solid phase providing loaded building block-linker construct (**9a**) as follows:

Building block-linker construct (**8a**) (1.0mmole), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluoro phosphate (HBTU, 379mg, 1.0mmole), 1-hydroxybenzotriazole.hydrate and (HOBT, 153mg, 1.0mmole) were dissolved in dimethylformamide (5mL) and N-methylmorpholine (NMM, 220μL, 2.0mmole) added. After pre-activation for 5 minutes, free amine gears (250 x 1.2μmole) were added, followed by dimethylformamide (30mL) and left overnight. The spent coupling solution was then added to free amine crowns (25 x 10μmole) and left overnight. Standard washing and analyses indicated loading at >95%.

The required ether carboxylic acid building block 2*RS*-Benzyloxy-3-cyclohexyl-propionic acid (compound (**12**), Scheme 3) was prepared as follows:

(1) Preparation of 3-Cyclohexyl-2*S*-hydroxypropionic acid (Compound (**11**) Scheme 3)

A solution of sodium nitrite (12.1g, 175mmol) in water (40ml) was added dropwise to a stirred suspension of (*S*)- α -aminocyclohexanepropionic acid hydrate (5g, 26.5mmol) in 0.5M sulphuric acid (120ml, 60mmol) at 0°C over 1.5hours. The mixture was allowed to warm to ambient temperature over 20hours. The product was
5 extracted into diethyl ether (2 x 25ml) then the ethereal layers were washed with saturated aqueous sodium chloride solution (2 x 25ml), dried (Na₂SO₄) and the solvents removed *in vacuo*. The residue (5.3g) was recrystallized from diethyl ether (10ml) and heptane (25ml) to give 3-cyclohexyl-2*S*-hydroxypropionic acid as a white solid, yield 2.4 g, (53%).

10

δ_H (400MHz, CDCl₃ at 298K), 0.89-1.35 (5H, m) and 1.51-1.86 (7H, m) (OCHCH₂ and cyclohexyl), 4.32 (1H, OCHCH₂, m)

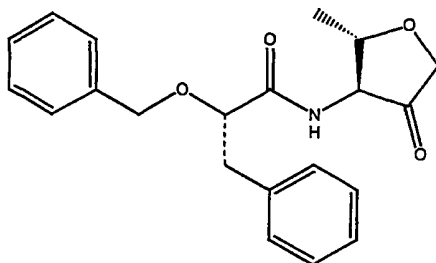
(2) Preparation of 2*RS*-Benzyloxy-3-cyclohexylpropionic acid (Compound (12)
15 Scheme 3)

Sodium hydride (265mg of 60% dispersion in oil, 6.6mmol) was added in two portions to a stirred mixture of 3-cyclohexyl-2*S*-hydroxypropionic acid (0.52g, 3.0mmol), dimethylformamide (5ml) and dichloromethane (5ml) at 0°C over
20 5minutes. The mixture was stirred at 0°C for 5minutes then at ambient temperature for 45minutes. Benzyl bromide (0.45ml, 3.8mmol) was added then the mixture stirred for 1 hour before adding dimethylformamide (5ml). After stirring for 4 hours potassium iodide (50mg, 0.3mmol) was added. The mixture was stirred for 20hours then heated at 55°C for 1hour then allowed to cool to ambient temperature and
25 poured into water (15ml). A saturated aqueous sodium chloride solution (5ml) was added then the mixture was extracted with dichloromethane (5ml then 10ml) that was discarded. The aqueous layer was acidified using 1M hydrochloric acid (10 ml) then extracted with dichloromethane (2 x 10ml). The dichloromethane layer was dried (MgSO₄) and the solvent removed *in vacuo*. The residue (0.55g) was dissolved in
30 dimethylformamide (8ml) then cooled to 0°C before adding sodium hydride (190mg of 60% dispersion in oil, 4.75mmol). The mixture was stirred for 30 minutes then polymer bound isocyanate (380mg, 2mmolNg⁻¹) added. The mixture was stirred for 2

- hours at ambient temperature then poured into water (15ml). 1M Hydrochloric acid (10ml) was added then the product was extracted into dichloromethane (2 x 10ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane
- 5 0:1 \rightarrow 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to give 2*RS*-benzyloxy-3-cyclohexylpropionic acid as a colourless oil, yield 41mg (5.2%). HPLC-MS (single main UV peak with $R_t = 9.47$ mins, 261.2 $[\text{M} - \text{H}]^-$, 285.2 $[\text{M} + \text{Na}]^+$, 547.3 $[2\text{M} + \text{Na}]^+$).
- 10 δ_{H} (400MHz, CDCl_3 at 298K), 0.72-1.03 (2H, cyclohexane, m), 1.08-1.38 (3H, cyclohexane, m), 1.45-1.93 (6H + 2H β , cyclohexane, m), 3.93-4.18 (1H α , OCHCO), 4.35-4.53 (1H, CH_2O , d, $J = 11.52$ Hz), 4.68-4.88 (1H, CH_2O , d, $J = 11.54$ Hz), 7.20-7.47 (5H, ArH, m), 9.36 (1H, OH, brs).
- 15 Compound (12) was coupled under standard conditions to loaded building block-linker construct (9a) (following standard removal of Fmoc), then cleaved to provide EXAMPLE 1. The crude example was analysed (see general techniques). HPLC $R_t = 20.74$ mins (> 90 %), HPLC-MS 360.2 $[\text{M} + \text{H}]^+$, 741.4 $[2\text{M} + \text{Na}]^+$.
- 20 The following examples (2 – 10) were prepared as detailed for EXAMPLE 1, coupling with the required carboxylic acid building blocks to provide the full length molecule.

EXAMPLE 2 . (2*S*, 3*S*) 2-Benzyloxy-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-3-phenyl-propionamide

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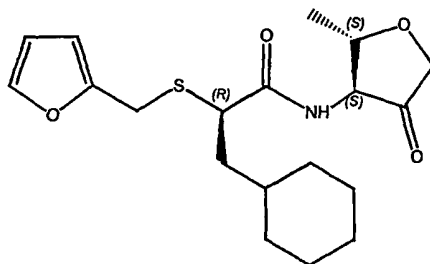
HPLC Rt = 18.34mins (> 90 %), HPLC-MS 354.2 $[M + H]^+$, 729.3 $[2M + Na]^+$.

The required carboxylic acid, 2*RS*-benzyloxy-3-phenylpropionic acid was prepared following the general method described for compound (12) as a colourless oil, yield
5 62 mg. HPLC-MS (single main UV peak with Rt = 8.27mins, 257.1 $[M + H]^+$).

1H ($CDCl_3$ at 298K); 3.05-3.30 (2H, $PhCH_2$, m), 4.15-4.30 (1H, OCH_2CO , m), 4.36-4.50 (1H, CH_2O , d, $J = 11.83$ Hz), 4.66-4.83 (1H, CH_2O , d, $J = 11.85$ Hz), 7.13-7.45 (10H, ArH, m), 10.07 (1H, OH, brs).

10

EXAMPLE 3. (2*S*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



15 HPLC Rt = 19.74 mins (> 80 %), HPLC-MS 366.1 $[M + H]^+$, 753.2 $[M + Na]^+$.

The required ether carboxylic acid building block 3-Cyclohexyl-2*R*-(furan-2-ylmethylsulfanyl)-propionic acid (compound (16), Scheme 4) was prepared as follows:

20

(1) Preparation of (*S*)- α -bromocyclohexanepropionic acid

A solution of sodium nitrite (2.26 g, 32.7mmol) in water (6 ml) was added drop-wise at 0°C over 4 hours to a stirred mixture of (*S*)- α -aminocyclohexanepropionic acid
25 (5g, 29.2mmol), potassium bromide (11.40g, 95.8mmol) and concentrated sulphuric acid (3.3 ml) in water (35 ml). The mixture was stirred for 5 hours at 0°C then at ambient temperature for 16 hours. The product was extracted into diethyl ether (4 x 50 ml) then the combined ethereal layers were washed with saturated aqueous sodium chloride solution (2 x 50 ml), dried ($MgSO_4$) and the solvent removed *in*

vacuo. The residue was purified by chromatography over silica gel eluting with a gradient of methanol : dichloromethane 1:50 \rightarrow 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (*S*)- α -bromocyclohexanepropionic acid as a pale yellow oil (3.64 g, 53%). TLC (single spot, R_f = 0.45, 10% methanol in dichloromethane), HPLC-MS (single main peak with R_t = 8.85mins, 234.1 / 236.1 $[M + H]^+$).

1H ($CDCl_3$ at 298K); 0.83-1.88 (11H, \underline{CH} (cyclohexane), $\underline{CH_2}$ (cyclohexane), m), 1.89-2.05 (2H, BrCH $\underline{CH_2}$, m), 4.32-4.44 (1H, BrCH $\underline{CH_2}$ (cyclohexane), m).
 ^{13}C ($CDCl_3$ at 298K); 26.19/26.29/26.48/26.58/26.79, 32.51 and 34.28 (cyclohexane $\underline{CH_2}$), 33.32 ($\underline{CHCH_2CHCO}$), 42.40 (BrCH $\underline{CH_2}$), 68.83 (Br \underline{CH}), 176.01 (\underline{CO}).

(2)Preparation of 3-Cyclohexyl-2*R*-(furan-2-ylmethylsulfanyl)-propionic acid (compound (16), Scheme 4)

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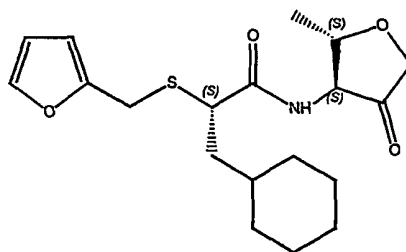
(*S*)- α -bromocyclohexanepropionic acid (0.41 g, 1.75mmol) and furan-2-ylmethylthiol (0.2 g, 1.75mmol) were dissolved in dimethylformamide (10 ml) and purged with nitrogen for 10 minutes. The solution was cooled to 0°C then triethylamine (0.244 ml, 1.75mmol) was added drop-wise over 1 minute. The mixture was stirred at 0°C for 30 minutes then at ambient temperature for 16 hours. The solvent was removed *in vacuo*, and the residue purified by chromatography over gel silica using methanol : dichloromethane 1:100 as eluent. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 3-Cyclohexyl-2*R*-(furan-2-ylmethylsulfanyl)-propionic acid (16) as a light brown oil (86mg, 18%). Analytical HPLC peak R_t = 18.68mins. TLC (single spot, R_f = 0.45, 10% methanol in dichloromethane), HPLC-MS (single main peak with R_t = 9.43mins, 291.1 $[M + Na]^+$).

1H ($CDCl_3$ at 298K); 0.67-0.88 (2H, $\underline{CH_2}$ (cyclohexane), m), 0.98-1.76 (11H, \underline{CH} (cyclohexane), $\underline{CH_2}$ (cyclohexane), m), 3.20-3.32 (1H, SCH \underline{CO} , t, J = 7.8 Hz), 3.69-3.79 (1H, $\underline{CH_2S}$, d, J = 14.7 Hz), 3.85-3.95 (1H, $\underline{CH_2S}$, d, J = 14.7 Hz), 6.14 / 6.28 (2H, furan H-3 and H-4, d), 7.30 (1H, furan H-5, s).

δ C (CDCl₃ at 298K); 26.26/26.45/26.58/26.77/26.89, 28.66, 33.11 and 33.34 (each d, cyclohexane $\underline{\text{C}}\text{H}_2$ and $\text{SCH}\underline{\text{C}}\text{H}_2$), 35.52 (u, $\underline{\text{C}}\text{HCH}_2\text{CHS}$), 38.49 (d, SCH_2), 43.84 (u, SCH), 108.64 (u, furan C-3), 110.78 (u, furan C-4), 142.65(u, furan C-5), 150.02 (q, furan C-2), 177.63 (q, $\underline{\text{C}}\text{O}$).

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EXAMPLE 4 (2*S*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



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HPLC Rt = 19.74 mins (> 80 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [M + Na]⁺.

The required ether carboxylic acid building block 3-Cyclohexyl-2*S*-(furan-2-ylmethylsulfanyl)-propionic acid (inverted isomer of compound (16), Scheme 4) was prepared as follows:

15

(1)Preparation of (*R*)- α -bromocyclohexanepropionic acid

A solution of sodium nitrite (0.45g, 6.5mmol) in water (1.2ml) was added dropwise at 0°C over 4 hours to a stirred mixture of (*R*)- α -aminocyclohexanepropionic acid (1g, 5.8mmol), potassium bromide (2.3g, 19.2mmol) and concentrated sulphuric acid (0.66ml) in water (7ml). The mixture was stirred for 5hours at 0°C then at ambient temperature for 16hours. The product was extracted into diethyl ether (4 x 20ml) then the combined ethereal layers were washed with saturated aqueous sodium chloride solution (2 x 50ml), dried (MgSO₄) and the solvent removed *in vacuo*. The residue was purified by chromatography over silica gel eluting with a gradient of methanol : dichloromethane 1:50 \rightarrow 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to give (*R*)- α -bromocyclohexanepropionic acid as a pale yellow oil 0.374 g, (27%). TLC (single spot, R_f = 0.45, 10% methanol in

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dichloromethane), HPLC-MS (single main peak with $R_t = 8.88$ mins, 234.1 / 236.1 $[M + H]^+$, 257.2 / 259.2 $[M + Na]^+$).

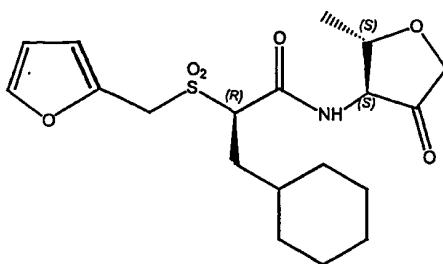
δ_H (400MHz, $CDCl_3$ at 298K), 0.83-1.88 (11H, \underline{CH} (cyclohexane), $\underline{CH_2}$ (cyclohexane), m), 1.89-2.05 (2H β , m), 4.32-4.44 (1H α , m).

(2)Preparation of 3-Cyclohexyl-2*S*-(furan-2-ylmethylsulfanyl)-propionic acid (inverted isomer of compound (16), Scheme 4)

(*R*)- α -bromocyclohexanepropionic (0.37g, 1.58mmol) and furan-2-yl methanethiol (0.18g, 1.58mmol) were dissolved in dimethylformamide (10ml) and purged with nitrogen for 10minutes. The solution was cooled to 0°C then triethylamine (0.22ml, 1.58mmol) was added drop-wise over 1 minute. The mixture was stirred at 0°C for 30minutes then at ambient temperature for 16hours. The solvent was removed *in vacuo*, and the residue purified by chromatography over gel silica using methanol : dichloromethane 1:100 as eluent. Appropriate fractions were combined and the solvents removed *in vacuo* to give 3-Cyclohexyl-2*S*-(furan-2-ylmethylsulfanyl)-propionic acid as a light brown oil, yield 142mg, (33%). Analytical HPLC peak $R_t = 18.68$ mins. TLC (single spot, $R_f = 0.45$, 10% methanol in dichloromethane), HPLC-MS (single main peak with $R_t = 9.53$ mins, 291.0 $[M + Na]^+$).

δ_H (400MHz, $CDCl_3$ at 298K), 0.68-0.89 (2H, $\underline{CH_2}$ (cyclohexane), m), 0.97-1.77 (11H, \underline{CH} (cyclohexane), $\underline{CH_2}$ (cyclohexane), m), 3.21-3.32 (1H α , t, $J = 7.8$ Hz), 3.69-3.79 (1H, $\underline{CH_2S}$, d, $J = 14.8$ Hz), 3.84-3.94 (1H, $\underline{CH_2S}$, d, $J = 14.8$ Hz), 6.15 / 6.28 (2H, furan H-3 and H-4), 7.30 (1H, furan H-5, s).

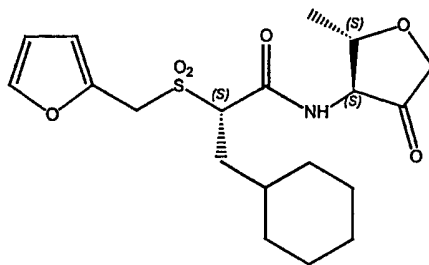
EXAMPLE 5. (2*S*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



HPLC Rt = 17.60 mins (> 80 %), HPLC-MS 398.1 [M + H]⁺, 817.1 [M + Na]⁺.

The intermediate loaded thioether (1.2μmole gear) of EXAMPLE 3 was oxidised
5 (Scheme 8) with m-chloroperbenzoic acid (5eq, 65% reagent, 1.6mg) in
dichloromethane (200μL) for 5hrs, followed by standard washing and then cleaved to
provide EXAMPLE 5.

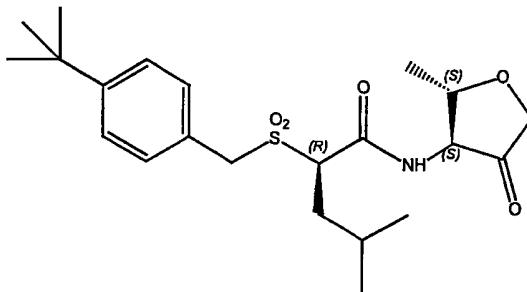
EXAMPLE 6. (2*S*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-
10 oxo-tetrahydrofuran-3-yl)-propionamide



HPLC Rt = 17.62 mins (> 95 %), HPLC-MS 398.1 [M + H]⁺, 817.1 [M + Na]⁺.

The intermediate loaded thioether (1.2μmole gear) of EXAMPLE 4 was oxidised
15 (Scheme 8) with m-chloroperbenzoic acid (5eq, 65% reagent, 1.6mg) in
dichloromethane (200μL) for 5hrs, followed by standard washing and then cleaved to
provide EXAMPLE 6.

EXAMPLE 7. (2*S*, 3*S*) 2-(4-*tert*-Butyl-phenylmethanesulfonyl)-4-methyl-pentanoic
20 acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



HPLC R_t = 21.14 mins (> 95 %), HPLC-MS 424.2 $[M + H]^+$, 869.4 $[2M + Na]^+$.

The required carboxylic acid building block 2*R*-(4-*tert*-Butyl-benzylsulfanyl)-4-methyl-pentanoic acid (analogue of compound (16), Scheme 4) was prepared from
5 (S)-2-bromo-4-methylpentanoic acid as follows:

(1) Preparation of (S)-2-bromo-4-methylpentanoic acid

A solution of sodium nitrite (5.1 g, 73mmol) in water (15 ml) was added drop-wise at
10 0°C over 5 hours to a stirred mixture of L-leucine (8.75 g, 67mmol), potassium bromide (29.75 g, 0.25mol) and concentrated sulphuric acid (8.6 ml) in water (100 ml). The mixture was stirred for 30 minutes at 0°C then at ambient temperature for 20 hours. The product was extracted into diethyl ether (2 x 150 ml) then the combined ethereal layers were washed with saturated aqueous sodium chloride
15 solution (2 x 100 ml), dried (MgSO₄) and the solvent removed *in vacuo*. The residue was purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane 1:50 → 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (S)-2-bromo-4-methylpentanoic acid as a colourless oil (2.22 g, 17%). TLC (single spot, R_f = 0.2, methanol : dichloromethane
20 1:20).

1H (CDCl₃ at 298K); 0.95 and 0.99 (both 3H, $\underline{CH_3}CH$, d, J = 6.55Hz), 1.77-1.89 (1H, $\underline{CH_3}CH$, m), 1.93 (2H, $\underline{CH_2}CH$, m), 4.31 (1H, $\underline{CH}Br$, t, J = 7.7Hz), 9.3 (1H, $\underline{CO_2H}$, brs).

25 Additional (S)-2-bromo-4-methylpentanoic acid was obtained (6.55g), however other components were observed by TLC analysis (methanol : dichloromethane 1:20).

(2)Preparation of 2*R*-(4-*tert*-butylbenzylsulfanyl)-4-methylpentanoic acid

30 A solution of (S)-2-bromo-4-methylpentanoic acid (1.1 g, 5.6mmol) and [4-(*tert*-butyl)phenyl]methanethiol (1.0 g, 5.6mmol) in dimethylformamide (15 ml) was purged with nitrogen for 5 minutes then cooled to 0°C. Triethylamine (0.79 ml, 5.7mmol) was added drop-wise over 1 minute then the mixture was stirred for two

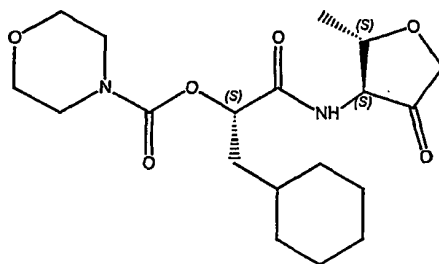
days at ambient temperature. The solvents were removed *in vacuo* and residue purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane 0:1 \rightarrow 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to leave a residue which was purified by flash chromatography over silica gel eluting with ethyl acetate : heptane 2:5. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 2*R*-(4-*tert*-butylbenzylsulfanyl)-4-methylpentanoic acid as a colourless oil (200 mg, 12%). TLC (single spot, R_f = 0.2, heptane : ethyl acetate 5:2), analytical HPLC with main peak R_t = 22.117mins, HPLC-MS (main UV peak with R_t = 11.072mins, 317.2 $[M + Na]^+$).

1H ($CDCl_3$ at 298K); 0.70 and 0.85 (both 3H, CH_3CH , d, J = 6.3), 1.29 (9H, $(CH_3)_3C$, s), 1.44-1.51 (1H, CH_3CH , m), 1.62-1.75 (2H, CH_2CH , m), 3.15-3.20 (1H, SCH_2 , m), 3.81 and 3.88 (both 1H, SCH_2 , d, J = 13.2Hz), 7.25-7.35 (4H, aromatic).

15

The intermediate loaded thioether (1.2 μ mole gear) was oxidised (Scheme 8) with *m*-chloroperbenzoic acid (5eq, 65% reagent, 1.6mg) in dichloromethane (200 μ L) for 5hrs, followed by standard washing and then cleaved to provide EXAMPLE 7.

20 EXAMPLE 8. (2*S*, 3*S*) Morpholine-4-carboxylic acid 2-cyclohexyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester



HPLC-MS 383.4 $[M + H]^+$.

25 The required carboxylic acid building block Morpholine-4-carboxylic acid 1-carboxy-2-cyclohexyl-ethyl ester (compound (19), Scheme 5) was prepared as follows:

(1) Preparation of 3-cyclohexyl-2S-hydroxypropionic acid methyl ester. (Compound (18), Scheme 5)

Trimethylsilyl chloride (1.78ml, 14.0mmol) was added dropwise to a stirred solution of 3-cyclohexyl-2S-hydroxypropionic acid (compound (11) 1.31g, 7.6mmol) in methanol (35ml). The mixture was stirred at room temperature for 20hours then the solvents were removed *in vacuo* to leave a pale yellow oil (1.38g). The crude oil was purified by chromatography over silica gel eluting with a gradient of n-heptane : ethyl acetate (4:1) Appropriate fractions were combined and the solvents removed *in vacuo* to give 3-cyclohexyl-2S-hydroxypropionic acid methyl ester as a colourless oil, yield 1.26 g, (89%). HPLC-MS (single main peak with $R_t = 7.43$ mins, 187.14 [M + H]⁺, 395.2 [2M + Na]⁺).

δ_H (400MHz, CDCl₃ at 298K), 0.84-1.04 (2H, CH₂(cyclohexane), m), 1.09-1.36 (3H, CH₂(cyclohexane), CH (cyclohexane), m), 1.45-1.77 (7H, CH₂(cyclohexane), CH(cyclohexane), m), 1.80-1.90 (1H β , m), 2.72 (1H, OH, d, $J = 5.99$ Hz), 3.80 (3H, CH₃O, s), 4.20-4.31 (1H α , m).

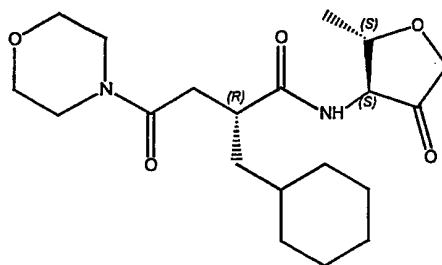
(2)Preparation of Morpholine-4-carboxylic acid 1-carboxy-2-cyclohexylethyl ester (Compound (18), Scheme 5)

A solution of phosgene (33.6ml, 20% in toluene) was added to 3-cyclohexyl-2S-hydroxypropionic acid methyl ester (compound (11), 0.7 g, 4.03mmol) followed by 6 drops of dimethylformamide. The mixture was stirred at ambient temperature for 16hours then the solvents removed *in vacuo*. The residue was azeotroped with toluene (3 x 20ml), and dissolved in anhydrous dichloromethane (11ml). The solution was cooled to 0°C then morpholine (0.86g, 9.85mmol) added. The mixture was stirred for 2 hours then partitioned between dichloromethane (30ml) and 0.5M hydrochloric acid (30ml). The dichloromethane layer was washed with saturated aqueous sodium hydrogen carbonate solution (30ml), saturated aqueous sodium chloride solution (30ml), dried (Na₂SO₄) and the solvent removed *in vacuo*. The residue was purified by chromatography over silica gel eluting with ethyl acetate :

heptane 1:1. Appropriate fractions were combined and the solvents removed *in vacuo* to leave morpholine-4-carboxylic acid 2*S*-cyclohexyl-1-methoxycarbonylethyl ester (0.13g, 10%) as an oil. A solution of lithium hydroxide monohydrate (17.5mg, 0.418mmol) in water (0.76ml) was added to an iced-water chilled solution of morpholine-4-carboxylic acid 2-cyclohexyl-1-methoxycarbonylethyl ester (110mg, 0.367mmol) in dioxane (1.5ml). The mixture was stirred at ambient temperature for 1 hour then diluted with water (10ml). The aqueous layer was extracted with diethyl ether (2 x 10ml) which was discarded, then acidified to pH = 2 with 6M hydrochloric acid. The product was extracted into diethyl ether (2 x 10ml), then the combined ethereal layers washed with saturated aqueous sodium chloride (10ml), dried (MgSO₄) and the solvent removed *in vacuo* to give morpholine-4-carboxylic acid 1-carboxy-2-cyclohexylethyl ester as a white solid, yield 0.11g, (100% from ester). TLC (single spot, R_f = 0.20, methanol : dichloromethane 1:9), HPLC-MS (single main peak with R_t = 7.614mins, 286.2 [M + H]⁺, 287.2 [M + 2H]⁺, 593.3 [2M + Na]⁺).

δ_H (400MHz, CDCl₃ at 298K), 0.75-1.00 (2H, CH₂(cyclohexane), m), 1.02-1.28 (4H, CH₂(cyclohexane), m), 1.33-1.46 (1H, CH(cyclohexane), m), 1.50-1.79 (6H, CH₂(cyclohexane), m), 3.28-3.73 (8H, CH₂OCH₂ and CH₂NCH₂, m), 4.92-5.02 (1H α , m), 5.99 (1H, OH, brs).

EXAMPLE 9. (2*S*, 3*S*) 2-Cyclohexylmethyl-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-4-morpholin-4-yl-4-oxo-butyramide



HPLC-MS 381.4 [M + H]⁺.

The required carboxylic acid building 2*R*-cyclohexylmethyl-4-morpholin-4-yl-4-oxo-butyric acid (compound (22), Scheme 6) was prepared as follows:

5 (1) Preparation of 2*R*-cyclohexylmethyl-4-morpholin-4-yl-4-oxo-butyric acid methyl ester.

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.HCl (1.12g, 5.69mmol) then 1-hydroxybenzotriazole monohydrate (0.87g, 5.69mmol) were added to a stirred solution of 2*R*-(cyclohexylmethyl)succinic acid 1-methyl ester (compound (21), 1.0g, 10 4.38mmol) in dimethylformamide (10ml) at 0°C under argon. The mixture was stirred for 25 minutes then morpholine (0.7 ml, 8.76mmol) was added drop-wise over 1 minute and stirring continued at ambient temperature for 16 hours. The product was extracted into ethyl acetate (200ml) then washed with 1.0M hydrochloric acid (3 x 100ml), saturated aqueous sodium hydrogen carbonate solution (3 x 100ml), water 15 (100ml), then saturated aqueous sodium chloride solution (100ml), dried (MgSO₄), and the solvent removed *in vacuo* to give 2*R*-cyclohexylmethyl-4-morpholin-4-yl-4-oxo-butyric acid methyl ester as an off-white solid, yield 1.22g, (94%). HPLC-MS (single peak with Rt = 7.91mins, 298.1 [M + H]⁺, 617.3 [2M + Na]⁺).

20 (2) Preparation of 2*R*-cyclohexylmethyl-4-morpholin-4-yl-4-oxo-butyric acid (Compound (22), Scheme 6).

A solution of lithium hydroxide monohydrate (0.51g, 12.18mmol) in water (27ml) was added a stirred solution of 2*R*-cyclohexylmethyl-4-morpholin-4-yl-4-oxo-butyric acid methyl ester (1.21g, 4.06mmol) in tetrahydrofuran (55ml) and methanol (27ml) 25 at 0°C. The mixture was stirred at ambient temperature for 1 hours then diluted with water (100ml). The aqueous layer was extracted with diethyl ether (2 x 50ml) which was discarded, then acidified to pH = 1-2 with 1M hydrochloric acid. The product was extracted into dichloromethane (3 x 50ml), then the combined ethereal layers 30 washed with water (2 x 50ml), saturated aqueous sodium chloride solution (2 x 50ml), dried (MgSO₄) and the solvent removed *in vacuo* to leave a residue. The residue was purified by chromatography over silica gel eluting with a gradient of

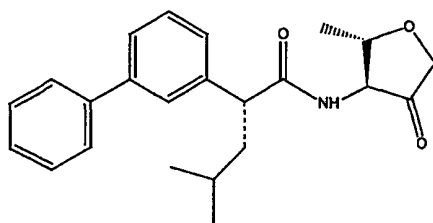
methanol : dichloromethane 1:100 \rightarrow 3:100. Appropriate fractions were combined and the solvents removed *in vacuo* was to give 2*R*-cyclohexylmethyl-4-morpholin-4-yl-4-oxo-butyric acid (**22**) as a white solid, yield 0.82 g, (71%). HPLC-MS (single peak with $R_t = 6.769$ mins, 284.2 $[M + H]^+$, 589.2 $[2M + Na]^+$).

5

δ_H (400MHz, $CDCl_3$ at 298K), 0.77-0.90 (2H, $\underline{CH_2}$ (cyclohexane), m), 1.05-1.40 (4H, $\underline{CH_2}$ (cyclohexane),m), 1.50-1.90 (7H, \underline{CH} (cyclohexane), $\underline{CH_2}$ (cyclohexane), m), 2.30-2.44 (2H β , m), 2.64-2.77 (1H α , m), 2.96-3.10 (1H, \underline{OH} , brs), 3.40-3.78 (8H, $\underline{CH_2OCH_2}$ and $\underline{CH_2NCH_2}$, m).

10

EXAMPLE 10. (2*S*, 3*S*) 2-Biphenyl-3-yl-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



15 HPLC $R_t = 20.60$ mins ($> 95\%$), HPLC-MS 366.1 $[M + H]^+$, 753.2 $[2M + Na]^+$.

The required carboxylic acid building block 2*RS*-Biphenyl-3-yl-4-methylpentanoic acid (compound (**25**), Scheme 7) was prepared as follows

20 (1)Preparation of Biphenyl-3-yl-acetic acid methyl ester (Compound (**24**), Scheme 7)

Concentrated sulphuric acid (588 μ L) was added to a solution of 3-bromophenyl acetic acid (10g, 46.5mmol) in methanol (100mL). The mixture was refluxed for 1.5h and then cooled to ambient temperature and evaporated under reduced pressure to afford a residue. The residue was redissolved in diethyl ether (500mL), washed with water (2 x 100mL), brine (100mL), dried ($MgSO_4$) and then evaporated under reduced pressure to afford 3-bromophenyl acetic acid methyl ester (10.65g). The 3-

bromophenyl acetic acid methyl ester was dissolved in toluene (117mL) then phenyl boronic acid (6.8g, 55.69mmol) added, followed by a aqueous solution of sodium carbonate (93mL, 2M) and tetrakis(triphenylphosphine)palladium (1.6g, 1.41mmol). The mixture was stirred overnight then cooled to ambient temperature and an
5 aqueous solution of saturated ammonium chloride (100mL) added. The mixture was extracted with ethyl acetate (2 x 200mL), dried (Na_2SO_4) and evaporated under reduced pressure to afford a residue. Flash chromatography of the residue over silica (200g) using ethyl acetate : heptane (3 : 48) as the eluent gave biphenyl-3-yl acetic acid methyl ester, yield 10.5g, (99 %), TLC (single UV spot, $R_f = 0.24$, 10% ethyl
10 acetate in heptane), analytical HPLC $R_t = 19.55$ min, HPLC-MS (single main UV peak with $R_t = 9.35$ min, 227.1 $[\text{M} + \text{H}]^+$).

δ_{H} (400MHz, CDCl_3 at 298K) 3.76 (2H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.77 (3H, s, OCH_3), 7.34-7.66 (9H, m, biphenyl-3-yl).

15

(2)Preparation of Biphenyl-3-yl-acetic acid

Water (39mL), followed by lithium hydroxide monohydrate (4.2g, 101.5mmol) were added to a solution of biphenyl-3-yl acetic acid methyl ester (11.43g, 50.57mmol) in
20 methanol (265mL). The mixture was stirred at ambient temperature for 2h then the organics were removed under reduced pressure. The mixture was acidified with dilute hydrochloric acid (1M, 80mL), extracted with chloroform (2 x 100mL), dried (MgSO_4) and evaporated under reduced pressure to afford biphenyl-3-yl acetic acid as a white solid, yield 10.6g, (99%), analytical HPLC $R_t = 16.565$ min, HPLC-MS
25 (single main UV peak with $R_t = 7.91$ min, 213.1 $[\text{M} + \text{H}]^+$).

δ_{H} (400MHz, CDCl_3 at 298K) 3.77 (2H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 7.28-7.52 (9H, m, biphenyl-3-yl).

30 (3)Preparation of 2*RS*-Biphenyl-3-yl-4-methylpent-4-enoic acid

A solution of biphenyl-3-yl acetic acid (7.0g, 33mmol) in anhydrous tetrahydrofuran (84mL) was added dropwise to a solution of lithium diisopropyl amide (36.4mL, 2M solution in hexanes) in anhydrous tetrahydrofuran (84mL) at -78°C . The mixture was allowed to warm to 0°C and stirred for 40 min. The mixture was then cooled to -78°C and 3-bromo-2-methylpropene (4.97mL) rapidly added. The mixture was stirred for 1 h at -78°C then water (28mL) added and the organics removed under reduced pressure. The mixture was then acidified with hydrochloric acid (6M, 14ml), extracted with ethyl acetate (3 x 100 ml), dried (MgSO_4) and evaporated under reduced pressure to afford a residue. Flash chromatography of the residue over silica (400g) using methanol : dichloromethane (3 : 97) as the eluent afforded impure 2-biphenyl-3-yl-4-methylpent-4-enoic acid (8.3 g). Flash chromatography over silica (400g) using methanol : dichloromethane (1.5 : 98.5) afforded pure 2-biphenyl-3-yl-4-methylpent-4-enoic acid, yield 5.27g, (60 %), TLC (single UV spot, $R_f = 0.28$, 5 % methanol in dichloromethane), analytical HPLC $R_t = 19.99$ min, HPLC-MS (single main UV peak with $R_t = 9.57$ min, 267.1 $[\text{M} + \text{H}]^+$).

δ_{H} (400MHz, CDCl_3 at 298K), 1.765 (3H, s, CH_3), 2.53 (1H, dd, $J = 6.6$ and 14.7 Hz, 3- H_1), 2.91 (1H, dd, $J = 8.9$ and 14.7 Hz, 3- H_1), 3.92 (1H, dd, $J = 6.6$ and 8.9 Hz, 2-H), 4.79 (2H, d, $J = 10.7$ Hz, 5- H_2), 7.30-7.62 (9H, m, biphenyl-3-yl).

(4)Preparation of 2*RS*-Biphenyl-3-yl-4-methylpentanoic acid (Compound (25), Scheme 7)

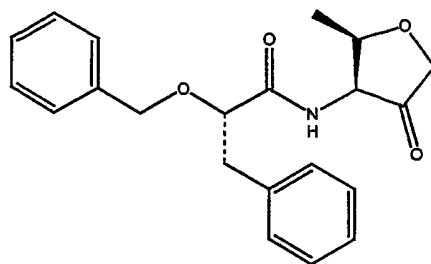
Palladium on carbon (10%, 300mg) was added portionwise to a solution of 2*RS*-biphenyl-3-yl-4-methylpent-4-enoic acid (1g, 3.76mmol) in ethanol (40mL) at 0°C . A hydrogen atmosphere was then introduced and the mixture allowed to warm to ambient temperature. The mixture was stirred for 18h, then the hydrogen atmosphere removed and the mixture filtered over Celite and the catalyst washed with ethanol (40mL). The combined organic filtrate was concentrated under reduced pressure to afford a residue, which was flash chromatographed over silica (150g) using methanol : dichloromethane (1 : 99) as the eluent to afford 2*RS*-biphenyl-3-yl-4-methylpentanoic acid, (25) yield 980mg, (98%), TLC (single UV spot, $R_f = 0.45$, 5 %

methanol in dichloromethane), analytical HPLC $R_t = 20.92$ min, HPLC-MS (single main UV peak with $R_t = 10.15$ min, 269.1 $[M + H]^+$, 291.1 $[M + Na]^+$).

δ_H (400MHz, $CDCl_3$ at 298K), 0.93 (6H, d, $J = 6.6$ Hz, 2 x CH_3), 1.52-1.57 (1H, m, 4- H_1), 1.71-1.76 (1H, m, 3- H_1), 1.97-2.05 (1H, m, 3- H_1), 3.66 (1H, t, $J = 7.8$ Hz, 2- H_1), 7.32-7.60 (9H, m, biphenyl-3-yl).

EXAMPLE 11 . (2*R*, 3*S*) 2-Benzyloxy-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-3-phenyl-propionamide

10

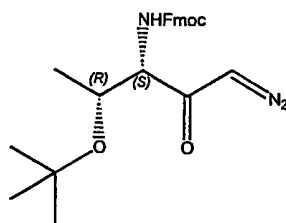


HPLC $R_t = 18.35$ mins ($> 90\%$), HPLC-MS 354.2 $[M + H]^+$, 729.3 $[2M + Na]^+$.

15 Following the general details from Scheme 1, the required bicycle building block (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9H-fluoren-9-ylmethyl ester (**6b**) was prepared as follows:

(1) Preparation of (1*S*, 1'*R*) [3-diazo-1-(1-*tert*-butoxyethyl)-2-oxo-propyl]carbamic acid 9H-fluoren-9-ylmethyl ester.

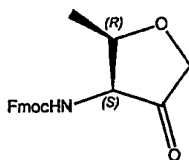
20



A solution of *iso*-butyl chloroformate (1.05 g, 7.7 mmol) in dichloromethane (14 ml) and a solution of 4-methylmorpholine (1.42 g, 14 mmol) in dichloromethane (14 ml)

were simultaneously added to a stirred suspension of Fmoc-*O*-(*tert*-butyl ether)-L-threonine (2.78g, 7mmol) in dichloromethane (70 ml) at -15 °C over 10 minutes under an atmosphere of nitrogen. Ethereal diazomethane [generated from diazald (7.14 g, ~21mmol) addition in diethyl ether (115 ml) to sodium hydroxide (8.022 g) in water (11 ml)/ethanol (31 ml) at 60°C] was then cautiously added and the resulting yellow solution was stirred at room temperature for 90 minutes. Acetic acid (~3 ml) was cautiously added (until effervescence had ceased) then the mixture was diluted with *tert*-butyl methyl ether (70 ml). The ethereal layer was washed with water (3 x 70 ml), dried (Na₂SO₄) and the solvent removed *in vacuo* to leave (1*S*, 1'*R*) [3-diazo-1-(1-*tert*-butoxyethyl)-2-oxo-propyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester as a yellow solid (4.28 g) which was used without further purification.

(2) Cyclisation to (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester.



A solution of lithium chloride (2.97 g, 70mmol) in 80% acetic acid in water (105 ml) was added to (1*S*, 1'*R*) [3-diazo-1-(1-*tert*-butoxyethyl)-2-oxo-propyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester (4.28 g, ~7mmol). The yellow oily suspension was stirred for 2 hours whereupon a pale yellow solution formed accompanied by the evolution of a gas. The solvents were removed *in vacuo* then the residue was dissolved in ethyl acetate (42 ml), washed with 10% aqueous sodium carbonate solution (2 x 42 ml) and saturated aqueous sodium chloride solution (42 ml). The combined aqueous layers were extracted with ethyl acetate (2 x 42 ml) then the combined ethyl acetate layers were dried (Na₂SO₄) and the solvents removed *in vacuo* to leave a yellow gum (3.44 g). The yellow gum was purified by chromatography over silica gel eluting with a gradient of n-heptane : ethyl acetate 9:1 → 7:3. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester as a white crystalline solid (2.92 g, 62% from starting acid). TLC

(single UV spot, $R_f = 0.27$, n-heptane : ethyl acetate 2:1), analytical HPLC peak $R_t = 17.96$ mins, HPLC-MS (single UV peak with $R_t = 8.48$ mins, $360.0 [M + Na]^+$, $361.0 [M + H + Na]^+$).

- 5 1H ($CDCl_3$ at 298K); 1.37-1.60 (3H, CH_3CHO , brs), 3.68-3.86 (1H, CH_3CHO m), 3.89-4.09 (2H, Fmoc CH_2 , m), 4.16-4.34 (2H, Fmoc H-9 and $NHCHCO$, m), 4.36-4.57 (2H, OCH_2CO , dd), 5.05-5.25 (1H, NH, brs), 7.28-7.47 (4H, ArH, Fmoc H-2 and H-7, ArH, Fmoc H-3 and H-6), 7.50-7.66 (2H, ArH, Fmoc H-1 and H-8), 7.73-7.85 (2H, ArH, Fmoc H-4 and H-5).
- 10 ^{13}C ($CDCl_3$ at 298K); 18.61 (u, CH_3CHO), 46.69 (u, Fmoc C-9), 62.26 (u, $NHCHCO$), 66.82 (d, Fmoc CH_2), 70.38 (d, $COCH_2$), 76.93 (u, CH_3CHO), 119.60 (u, Fmoc C-4 and C-5), 124.55 (u, Fmoc C-1 and C-8), 126.69 (u, Fmoc C-2 and C-7), 127.37 (u, Fmoc C-3 and C-6), 140.92 (q, Fmoc C-4' and C-5'), 143.15/143.20 (q, Fmoc C-1' and C-8'), 155.61 (q, $OCON$), 211.38 (q, $COCH_2$).

15

Following the general details from Scheme 2, the required bicycle building (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9H-fluoren-9-ylmethyl ester (**6b**) was converted to building block-linker construct (**8b**) as follows:

- 20 (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9H-fluoren-9-ylmethyl ester (**6b**) (354mg, 1.05mmol, 1 eq) was dissolved in a mixture of ethanol (9.6 mL) and water (1.4 mL) containing sodium acetate.trihydrate (218mg, 1.6 mmol, 1.5 eq). 4-[[[(hydrazinocarbonyl)amino] methyl]cyclohexanecarboxylic acid. trifluoroacetate (346mg, 1.05mmol, 1 eq, Murphy, A. M. *et al*, *J. Am. Chem. Soc.*,
25 114, 3156-3157, 1992) was added and the mixture refluxed for 2hr. Chloroform (100mL) was added and the organics washed with dilute aqueous hydrochloric acid (0.1 M, 1 x 100 mL). The acidic layer was backwashed with chloroform (3 x 100mL) and the combined organics washed with brine (100 mL), dried (Na_2SO_4) and evaporated under reduced pressure to afford linker construct (**8b**) as a white solid
30 (660mg). Analytical HPLC indicated one main peak at $R_t = 17.011$ min, HPLC-MS (main UV peak with $R_t = 7.96$ min, $535.3 [M + H]^+$. Crude (**8b**) was used directly for construct loading.

Following the general details from Scheme 2, the required building block-linker construct (**8b**) was attached to the solid phase providing loaded building block-linker construct (**9b**) as follows:

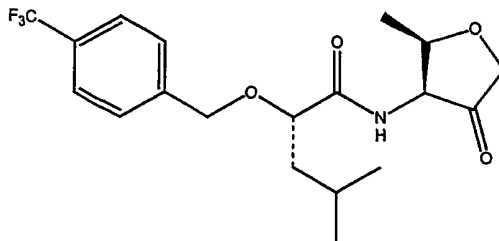
- 5 Building block-linker construct (**8b**) (1.0mmole), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluoro phosphate (HBTU, 379mg, 1.0mmole), 1-hydroxybenzotriazole.hydrate and (HOBT, 153mg, 1.0mmole) were dissolved in dimethylformamide (5mL) and N-methylmorpholine (NMM, 220 μ L, 2.0mmole) added. After pre-activation for 5 minutes, free amine gears (250 x 1.2 μ mole) were
 10 added, followed by dimethylformamide (30mL) and left overnight. The spent coupling solution was then added to free amine crowns (25 x 10 μ mole) and left overnight. Standard washing and analyses indicated loading at >95%.

- 2*RS*-benzyloxy-3-phenylpropionic acid was coupled under standard conditions to
 15 loaded building block-linker construct (**9b**) (following standard removal of Fmoc), then cleaved to provide EXAMPLE 11. The crude example was analysed (see general techniques). HPLC Rt = 18.35 mins (> 90 %), HPLC-MS 354.2 [M + H]⁺, 729.3 [2M + Na]⁺.

- 20 The following examples (**12 – 23**) were prepared as detailed for EXAMPLE 11, coupling with the required reagents to provide the full length molecule.

EXAMPLE 12 . (2*R*, 3*S*) 4-Methyl-2-(4-trifluoromethylbenzyloxy)-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide

25



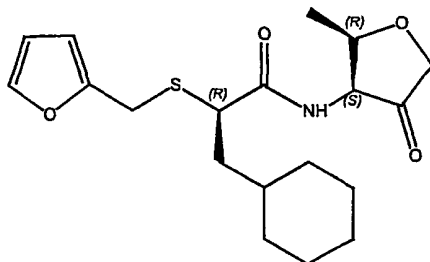
HPLC Rt = 20.83 mins (> 90 %), HPLC-MS 388.2 [M + H]⁺, 797.3 [2M + Na]⁺.

The required carboxylic acid building block 2*RS*-4-Methyl-2-(4-trifluoromethyl-benzyloxy)-pentanoic acid was prepared as follows

5 (1) Preparation of 2*RS*-4-Methyl-2-(4-trifluoromethyl-benzyloxy)-pentanoic acid

Sodium hydride (333 mg of 60% dispersion in oil, 8.3mmol) was added in two portions to a stirred mixture of (*S*)-2-hydroxy-4-methylpentanoic acid (0.5 g, 3.8mmol), dimethylformamide (5 ml) and dichloromethane (5 ml) at 0°C over 5
10 minutes. The mixture was stirred at 0°C for 5 minutes then at ambient temperature for 45 minutes. 4-(Trifluoromethyl)benzyl bromide (0.73 ml, 4.7mmol) was added then the mixture stirred for 5 hours before adding potassium iodide (50 mg, 0.3mmol) and dimethylformamide (5 ml). The mixture was stirred for 20 hours then heated at 55°C for 1 hour then allowed to cool to ambient temperature and poured
15 into water (15 ml). A saturated aqueous sodium chloride (5 ml) was added then the mixture was extracted with dichloromethane (5 ml then 10 ml) that was discarded. The aqueous layer was acidified using 1M hydrochloric acid (10 ml) then extracted with dichloromethane (2 x 10 ml). The dichloromethane layer was dried (MgSO₄) and the solvent removed *in vacuo*. The residue (0.52 g) was dissolved in
20 dimethylformamide (5 ml) then cooled to 0°C before adding sodium hydride (160 mg of 60% dispersion in oil, 4mmol). The mixture was stirred for 30 minutes then polymer bound isocyanate (320 mg, 2mmol Ng⁻¹) added. The mixture was stirred for 2 hours at ambient temperature then poured into water (15 ml). 1M Hydrochloric acid (10 ml) was added then the product was extracted into dichloromethane (2 x 10
25 ml), dried (Na₂SO₄) and the solvent removed *in vacuo*. The residue was purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane 0:1 → 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 2*RS*-4-Methyl-2-(4-trifluoromethylbenzyloxy)-pentanoic acid, yield 121 mg (11%) as a colourless oil. HPLC-MS (single peak with Rt =
30 9.66mins, 291.1 [M + H]⁺, 313.1 [M + Na]⁺).

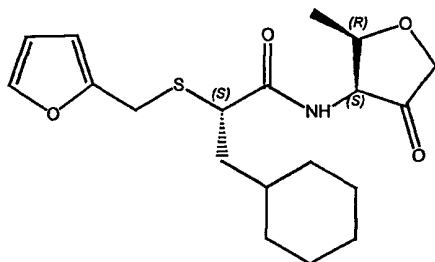
EXAMPLE 13. (2*R*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



- 5 HPLC Rt = 19.73 mins (> 80 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [M + Na]⁺.

Prepared as detailed for EXAMPLE 3, but using loaded building block-linker construct (9b)

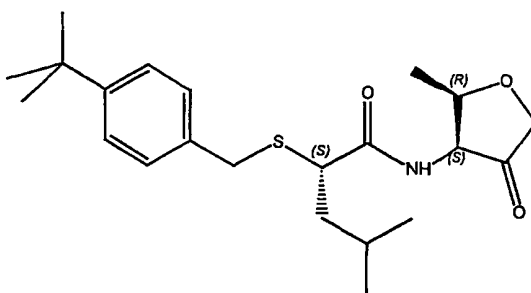
- 10 EXAMPLE 14. (2*R*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



HPLC Rt = 19.73 mins (> 80 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [M + Na]⁺.

- 15 Prepared as detailed for EXAMPLE 4, but using loaded building block-linker construct (9b)

EXAMPLE 15. (2*R*, 3*S*) 2-(4-*tert*-Butyl-benzylsulfanyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydro-furan-3-yl)-amide



HPLC Rt = 23.21 mins (> 95 %), HPLC-MS 392.2 [M + H]⁺, 805.4 [2M + Na]⁺.

The required carboxylic acid building block 2*S*-(4-*tert*-Butyl-benzylsulfanyl)-4-methyl-pentanoic acid (analogue of compound (16), Scheme 4) was prepared from
5 (*R*)-2-bromo-4-methylpentanoic acid as follows:

(1) Preparation of 2*R*-Bromo-4-methylpentanoic acid

10 A solution of sodium nitrite (5.1g, 73mmol) in water (15ml) was added drop-wise at 0°C over 5hours to a stirred mixture of D-leucine (8.75g, 67mmol), potassium bromide (29.75g, 0.25mol) and concentrated sulphuric acid (8.6ml) in water (100ml). The mixture was stirred for 30 minutes at 0°C then at ambient temperature for 20hours. The product was extracted into diethyl ether (2 x 150ml) then the combined
15 ethereal layers were washed with saturated aqueous sodium chloride solution (2 x 100ml), dried (MgSO₄) and the solvent removed *in vacuo*. The residue was purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane 1:50 → 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 2*R*-bromo-4-methylpentanoic acid as a colourless oil,
20 yield 1.60 g, (12.3%). TLC (single spot, R_f = 0.2, methanol : dichloromethane 1:20). Additionally, a second crop (5.2g, 40%) of slightly impure product was obtained

δ_H (400MHz, CDCl₃ at 298K), 0.95 and 0.99 (both 3H, CH₃CH, d, *J* = 6.55Hz),
1.77-1.89 (1H, CH₃CH, m), 1.93 (2H β , m), 4.31 (1H α , t, *J* = 7.7Hz), 9.3 (1H, CO₂H,
25 brs).

(2)Preparation of 2*S*-(4-*tert*-butylbenzylsulfanyl)-4-methylpentanoic acid (analogue of compound (16), Scheme 4)

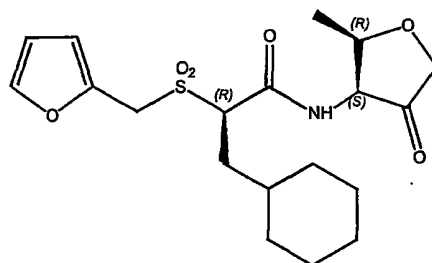
30 A solution of 2*R*-bromo-4-methylpentanoic acid (1.1g, 5.6mmol) and (4-(*tert*-butyl)phenyl)methanethiol (1.0g, 5.6mmol) in dimethylformamide (15ml) was purged with nitrogen for 5 minutes then cooled to 0°C. Triethylamine (0.79ml, 5.7mmol) was added drop-wise over 1 minute then the mixture was stirred for two

days at ambient temperature. The solvents were removed *in vacuo* and residue purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane 0:1 \rightarrow 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to leave a residue which was purified by flash chromatography over silica gel eluting with ethyl acetate : heptane 2:5. Appropriate fractions were combined and the solvents removed *in vacuo* to give 2*S*-(4-*tert*-butylbenzylsulfanyl)-4-methylpentanoic acid as a colourless oil, yield 150 mg, (9%). TLC (single spot, R_f = 0.2, heptane : ethyl acetate 5:2), analytical HPLC with main peak R_t = 22.117mins, HPLC-MS (main UV peak with R_t = 11.072mins, 317.2 $[M + Na]^+$).

10

δ_H (400MHz, $CDCl_3$ at 298K), 0.70 and 0.85 (both 3H, $\underline{CH_3}CH$, d, J = 6.3), 1.29 (9H, $(\underline{CH_3})_3C$, s), 1.44-1.51 (1H, CH_3CH , m), 1.62-1.75 (2H β , m), 3.15-3.20 (1H α , m), 3.81 and 3.88 (both 1H, SCH_2 , d, J = 13.2Hz), 7.25-7.35 (4H, aromatic).

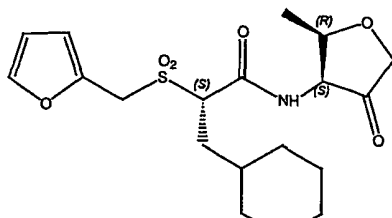
15 EXAMPLE 16. (2*R*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



HPLC R_t = 17.65 mins (> 80 %), HPLC-MS 398.1 $[M + H]^+$, 817.1 $[M + Na]^+$.

20 Prepared as detailed for EXAMPLE 5, but using loaded building block-linker construct (9b)

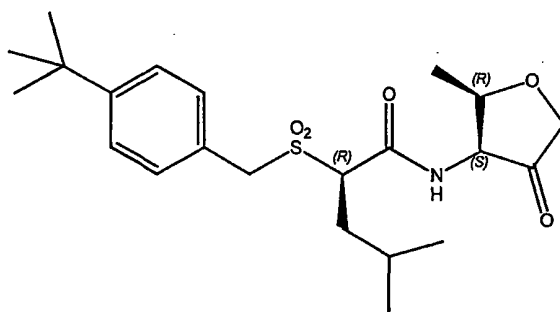
EXAMPLE 17. (2*R*, 3*S*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



HPLC Rt = 17.61 mins (> 95 %), HPLC-MS 398.1 [M + H]⁺, 817.1 [M + Na]⁺.

Prepared as detailed for EXAMPLE 6, but using loaded building block-linker
5 construct (9b)

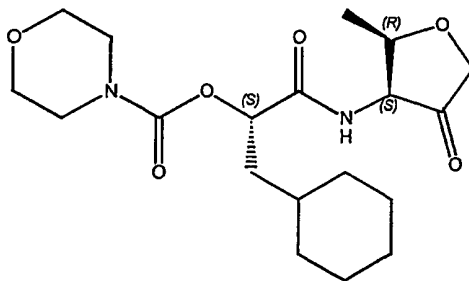
EXAMPLE 18. (2*R*, 3*S*) 2-(4-*tert*-Butyl-phenylmethanesulfonyl)-4-methyl-pentanoic
acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



10 HPLC Rt = 21.19 mins (> 95 %), HPLC-MS 424.2 [M + H]⁺, 869.4 [2M + Na]⁺.

Prepared as detailed for EXAMPLE 7, but using loaded building block-linker
construct (9b)

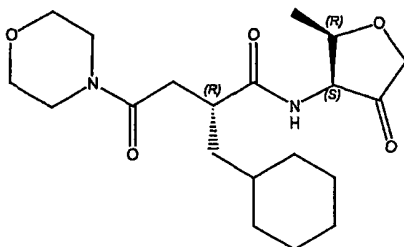
15 EXAMPLE 19. (2*R*, 3*S*) Morpholine-4-carboxylic acid 2-cyclohexyl-1-(2-methyl-4-
oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester



HPLC-MS 383.4 [M + H]⁺.

20 Prepared as detailed for EXAMPLE 8, but using loaded building block-linker
construct (9b)

EXAMPLE 20. (2*R*, 3*S*) 2-Cyclohexylmethyl-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-4-morpholin-4-yl-4-oxo-butyramide



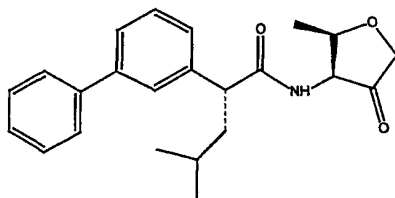
HPLC-MS 381.4 [M + H]⁺.

5

Prepared as detailed for EXAMPLE 9, but using loaded building block-linker construct (9b)

EXAMPLE 21. (2*R*, 3*S*) 2-Biphenyl-3-yl-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide

10

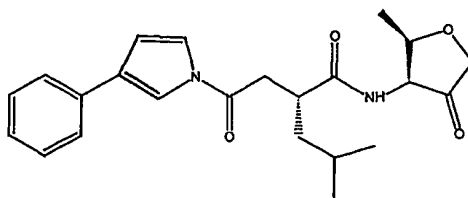


HPLC Rt = 20.62 mins (> 95 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [2M + Na]⁺.

15 Prepared as detailed for EXAMPLE 10, but using loaded building block-linker construct (9b)

EXAMPLE 22. (2*R*, 3*S*) 4-Methyl-2-[2-oxo-2-(3-phenyl-pyrrol-1-yl)-ethyl]-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide

20



HPLC Rt = 19.80 & 20.16mins (19.9 & 80.1%), HPLC-MS 397.2 $[M + H]^+$, 815.4 $[2M + Na]^+$.

- 5 The required carboxylic acid building block (2R) 4-Methyl-2-[2-oxo-2-(3-phenyl-pyrrol-1-yl)-ethyl]-pentanoic acid (analogue of compound (22), Scheme 6) was as follows:

(1) Preparation of 4-Phenyl-1H-pyrrole-3-carboxylic acid methyl ester

10

A solution of 4-toluenesulphonylmethyl isocyanide (4.9 g, 25.2mmol) and methyl trans-cinnamate (4.0 g, 24.7mmol) in diethyl ether (40 ml) and dimethyl sulphoxide (20 ml) was added drop-wise over 30 minutes to a stirred suspension of sodium hydride (60% dispersion, 1.25 g, 31.3mmol) in diethyl ether (100 ml) under nitrogen.

- 15 After 20 minutes a further portion of dimethyl sulphoxide (25 ml) was added to the reaction vessel. The mixture was stirred for 1 hour then water (200 ml) was cautiously added. The product was extracted into dichloromethane (1 x 400 ml and 1 x 250 ml) then the combined dichloromethane layers were dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was purified by flash chromatography over
20 silica gel eluting with a gradient of heptane : ethyl acetate : dichloromethane 2:1:0 → 1:1:1. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 4-phenyl-1H-pyrrole-3-carboxylic acid methyl ester as a white solid (1.55 g, 31%). TLC (single spot, R_f = 0.25, heptane : ethyl acetate 2:1), HPLC-MS (main UV peak with Rt = 7.849mins, 202.1 $[M + H]^+$, 425.1 $[2M + Na]^+$).

25

(2) Preparation of 3-Phenyl-1H-pyrrole

- A solution of potassium hydroxide (4.3 g, 77mmol) in water (40 ml) was added to a suspension of 4-phenyl-1H-pyrrole-3-carboxylic acid methyl ester (1.55 g, 7.7mmol)
30 in methanol (40 ml). The mixture was heated at reflux for 2.5 hours then the majority of solvents removed from the resulting solution by distillation *in vacuo*. The residue was diluted with water (100 ml) and ice (30 ml) added then the mixture was acidified

- with hydrochloric acid (1M) to pH=1. The precipitate was collected by filtration *in vacuo* then washed with water (75 ml), dichloromethane (75 ml), water (20 ml) then dichlororomethane (75 ml). The white solid was dried *in vacuo* then heated with ethanolamine (10 ml) at 190°C under an atmosphere of nitrogen for 2.25 hours. The
- 5 mixture was allowed to cool to ambient temperature then poured onto ice (150 ml). Water (50 ml) was added then the product was extracted into dichloromethane (3 x 50 ml). The dichloromethane layers were combined then dried (Na₂SO₄) and the solvent removed *in vacuo* to leave an oil (900 mg) which was purified by flash chromatography over silica gel eluting with ethyl acetate : heptane 1:5. Appropriate
- 10 fractions were combined and the solvents removed *in vacuo* to leave 3-phenyl-1H-pyrrole as a yellow-green oil which solidified after storage at -80°C for 20 hours (810 mg, 74%). TLC (single spot, R_f = 0.25, heptane : ethyl acetate 5:1), HPLC-MS (single UV peak with R_t = 8.493mins, 144.1 [M + H]⁺).
- 15 ¹H (CDCl₃ at 298K); 6.54, 6.80 and 7.06 (each 1H, pyrrole, m), 7.15-7.51 (5H, aromatic), 8.22 (1H, NH, brs)

(3) Preparation of (2R) Isobutyl-succinic acid

- 20 A solution of lithium hydroxide monohydrate (26 mg, 0.61mmol) in water (250μl) was added to a stirred solution of (2R) 2-isobutylsuccinic acid 1-methyl ester (100mg, 0.53mmol) in 1,4-dioxane (500μl). The mixture was stirred for 2 hours then lithium hydroxide monohydrate (26 mg, 0.61mmol) was added and stirring continued for 24 hours. The majority of solvents were removed by distillation *in vacuo* then the
- 25 residue was dissolved in water (5 ml) and acidified using hydrochloric acid (1M) to pH=1. The product was extracted into dichloromethane (5 x 5 ml), dried (Na₂SO₄) and the solvent removed *in vacuo* to obtain (2R) 2-isobutylsuccinic acid (49 mg, 53%) as a solid. HPLC-MS (single UV peak with R_t = 6.223mins, 197.1 [M + Na]⁺).
- 30 (4) Preparation of (2R) 4-Methyl-2-[2-oxo-2-(3-phenyl-pyrrol-1-yl)-ethyl]-pentanoic acid

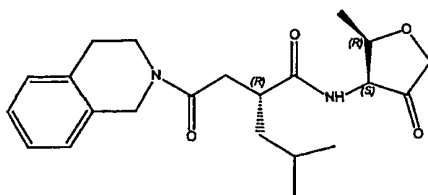
A solution of (2*R*)-2-isobutylsuccinic acid (35 mg, 0.2mmol) in acetyl chloride (1.2 ml) was heated at reflux under nitrogen for 2.2 hours then volatile components removed by distillation *in vacuo* to leave (3*R*)-3-isobutyldihydrofuran-2,5-dione as an oily residue which was used without further purification.

- 5 Sodium hydride (12 mg of 60% dispersion in oil, 0.3mmol) was added to a stirred solution of 3-phenylpyrrole (43 mg, 0.30mmol) in tetrahydrofuran (1.0ml) under nitrogen. The mixture was stirred for 15 minutes then added *via* cannula to a stirred solution of the (3*R*)-3-isobutyldihydrofuran-2,5-dione (0.2mmol) in tetrahydrofuran (1.0 ml) at 0°C, then stirred for 5 hours. The majority of solvents were removed *in*
- 10 *vacuo* then water (10 ml) added. The aqueous layer was extracted with ethyl acetate (5 ml) then saturated aqueous sodium chloride solution (5 ml) added and the aqueous layer extracted with an additional portion of ethyl acetate (5 ml). The aqueous layer was acidified using hydrochloric acid (1M) to pH=1 then the product extracted into ethyl acetate (2 x 5ml), dried (MgSO₄) and the solvent removed *in vacuo*. The
- 15 residue was purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane 1:99 → 3:17. Appropriate fractions were combined and the solvents removed *in vacuo* to leave a mixture (approximately 1:1) of (2*R*)-4-methyl-2-[oxo-2-(3-phenylpyrrol-1-yl)ethyl]pentanoic acid and (3*S*)- 5-Methyl-3-(3-phenyl-pyrrole-1-carbonyl)-hexanoic acid (1.6 mg, 2%) as an oil. TLC (two spots,
- 20 $R_f = 0.8$ and $R_f = 0.75$, methanol : dichloromethane 1:9), analytical HPLC two unresolved UV peaks with $R_t = 19.020$ and 19.127 mins, HPLC-MS (two unresolved UV peaks with $R_t = 9.724$ and 9.861 mins, $300.2 [M + H]^+$, $621.3 [2M + Na]^+$).

This mixture was used directly for the preparation of EXAMPLE 22.

25

EXAMPLE 23. (2*R*, 3*S*) 2-[2-(3,4-Dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



HPLC Rt = 16.03 & 16.53mins (> 80%), HPLC-MS 387.2 $[M + H]^+$, 795.4 $[2M + Na]^+$.

- 5 The required carboxylic acid building block (2R) 2-[2-(3,4-Dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid (analogue of compound (22), Scheme 6) was as follows:

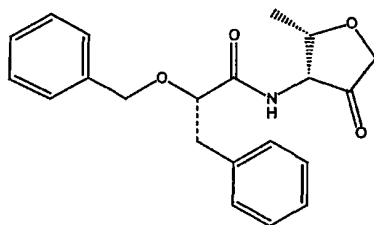
- (1) Preparation of 2-[2-(3,4-Dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-
10 pentanoic acid methyl ester.

- iso*-Butyl chloroformate (33.64 mg, 0.2463 mmol) in dichloromethane (2 ml) and *N*-methyl morpholine (49.83 mg, 0.4927 mmol) in dichloromethane (2 ml) were simultaneously added to a stirred solution of (*R*)-2-isobutyl succinic acid 1-methyl
15 ester (42.15 mg, 0.2239mmol) in dichloromethane (2 ml) at -15°C under argon over 5 minutes. The mixture was stirred at -15°C for 15 minutes. A freshly prepared solution of 1,2,3,4-tetrahydro-isoquinoline (29.83 mg, 0.2239mmol) in dichloromethane (2 ml) and *N*-methyldmorpholine (24.92 mg, 0.2463 mmol) was then added dropwise. The mixture was then stirred at ambient temperature for 16 hours.
- 20 The reaction mixture was poured into saturated aqueous sodium chloride solution (1 ml). The aqueous layer was extracted with diethyl ether (2 x 5 ml) which was discarded. The aqueous layer was acidified with 1M hydrochloric acid to pH (1~2), then the product was extracted into dichloromethane (3 x 5 ml). The combined dichloromethane layers were washed with water (2 x 5 ml), saturated aqueous
25 sodium chloride solution (2 x 5 ml) then dried (Na_2SO_4). The solvent was removed *in vacuo* to give a residue which was purified over silica gel eluting with a gradient of *n*-heptane : ethyl acetate 4:1. Desired fractions were combined and reduced *in vacuo* to leave 2-[2-(3,4-Dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid methyl ester as a colourless oil (35 mg, 51.5% from starting acid). TLC (single
30 UV spot, $R_f = 0.6$, *n*-heptane : ethyl acetate 1:1), HPLC-MS (single UV peak with Rt = 9.544mins, 304.2 $[M + H]^+$, 305.2 $[M + 2]^+$, 306.2 $[M + 3]^+$, 629.3 $[2M + Na]^+$).

(2) Preparation of 2-[2-(3,4-dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid.

- 5 A solution of lithium hydroxide monohydrate (0.01452 g, 0.3461mmol) in water (1.5 ml) was added drop-wise over 1 minute to a solution of 2-[2-(3,4-dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid methyl ester (0.035 g, 0.1154mmol) in tetrahydrofuran : methanol 2:1 (9 ml) at 0°C. The mixture was stirred at ambient temperature for 16 hours then poured into saturated aqueous sodium chloride solution (10 ml). The aqueous layer was extracted with diethyl ether (2 x 10 ml) which was discarded. The aqueous layer was acidified with 1M hydrochloric acid to pH (1~2), then the product was extracted into dichloromethane (3 x 10 ml). The combined dichloromethane layers were washed with water (2 x 10 ml), saturated aqueous sodium chloride solution (2 x 10 ml) then dried (Na₂SO₄) and the solvents removed *in vacuo* to leave 2-[2-(3,4-dihydro-1*H*-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid as a colourless oil (4.0mg, 12%). TLC (main spot, R_f = 0.2, methanol : dichloromethane 1:10), analytical HPLC R_t = 13.297 min. HPLC-MS (290.2 [M+1]⁺, 312.2 [M + Na]⁺, 601.3 [2M + Na]⁺).

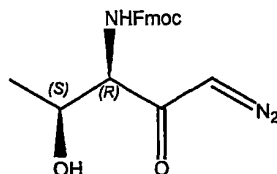
- 20 EXAMPLE 24. (2*S*, 3*R*) 2-Benzyloxy-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-3-phenyl-propionamide



- 25 HPLC R_t = 18.34 mins (> 90 %), HPLC-MS 354.2 [M + H]⁺.

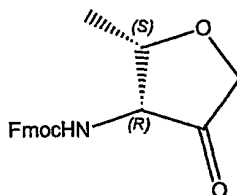
Following the general details from Scheme 1, the required bicycle building block (2*S*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (6c) was prepared as follows:

(1) Preparation of (1*R*, 1'*S*) [3-diazo-1-(1-hydroxyethyl)-2-oxo-propyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester.



A solution of *iso*-butyl chloroformate (1.05 g, 7.7 mmol) in dichloromethane (7 ml) and a solution of 4-methylmorpholine (1.42 g, 14 mmol) in dichloromethane (7 ml) were simultaneously added to a stirred suspension of Fmoc-D-threonine (2.39 g, 7mmol) in dichloromethane (70 ml) at -15 °C over 10 minutes under an atmosphere of nitrogen. Etheral diazomethane [generated from diazald (7.14 g, ~21mmol) addition in diethyl ether (115 ml) to sodium hydroxide (8.022 g) in water (11 ml)/ethanol (31 ml) at 60°C] was then cautiously added and the resulting yellow solution was stirred at room temperature for 90 minutes. Acetic acid (~3 ml) was cautiously added (until effervescence had ceased) then the mixture was diluted with *tert*-butyl methyl ether (70 ml). The etheral layer was washed with water (3 x 70 ml), dried (Na₂SO₄) and the solvent removed *in vacuo* to leave (1*R*, 1'*S*) [3-diazo-1-(1-hydroxyethyl)-2-oxo-propyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester as a yellow solid (3.43 g) which was used without further purification.

(2) Cyclisation to (2*S*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester.



20

A solution of lithium chloride (2.97 g, 70mmol) in 80% acetic acid in water (105 ml) was added to (1*R*, 1'*S*) [3-diazo-1-(1-hydroxyethyl)-2-oxo-propyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester (3.43 g, ~7mmol). The yellow oily suspension was stirred for 2 hours whereupon a pale yellow solution formed accompanied by the evolution of a gas. The solvents were removed *in vacuo* then the residue was dissolved in ethyl

25

acetate (42 ml), washed with 10% aqueous sodium carbonate solution (2 x 42 ml) and saturated aqueous sodium chloride solution (42 ml). The combined aqueous layers were extracted with ethyl acetate (2 x 42 ml) then the combined ethyl acetate layers were dried (Na_2SO_4) and the solvents removed *in vacuo* to leave a yellow gum (2.26 g). The yellow gum was purified by chromatography over silica gel eluting with a gradient of n-heptane : ethyl acetate 9:1 \rightarrow 7:3. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (2*S*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester as a white crystalline solid (659 mg, 28% from starting acid). TLC (single UV spot, R_f = 0.27, n-heptane : ethyl acetate 2:1), analytical HPLC peak R_t = 18.02mins, HPLC-MS (single UV peak with R_t = 8.47mins, 338.1 $[\text{M} + \text{H}]^+$, 360.1 $[\text{M} + \text{Na}]^+$, 361.0 $[\text{M} + \text{H} + \text{Na}]^+$).

^1H (CDCl_3 at 298K); 1.39-1.60 (3H, CH_3CHO , brs), 3.72-3.85 (1H, CH_3CHO m), 3.88-4.09 (2H, Fmoc CH_2 , m), 4.15-4.33 (2H, Fmoc H-9 and NHCHCO , m), 4.39-4.58 (2H, OCH_2CO , dd), 5.07-5.25 (1H, NH, brs), 7.28-7.38 (2H, ArH, Fmoc H-2 and H-7), 7.39-7.47 (2H, ArH, Fmoc H-3 and H-6), 7.52-7.66 (2H, ArH, Fmoc H-1 and H-8), 7.72-7.86 (2H, ArH, Fmoc H-4 and H-5).

^{13}C (CDCl_3 at 298K); 19.45 (u, CH_3CHO), 47.52 (u, Fmoc C-9), 63.09 (u, NHCHCO), 67.65 (d, Fmoc CH_2), 71.21 (d, COCH_2), 77.80 (u, CH_3CHO), 120.43 (u, Fmoc C-4 and C-5), 125.38 (u, Fmoc C-1 and C-8), 127.51 (u, Fmoc C-2 and C-7), 128.20 (u, Fmoc C-3 and C-6), 141.75 (q, Fmoc C-4' and C-5'), 143.98/144.03 (q, Fmoc C-1' and C-8'), 156.45 (OCON), 212.21 (COCH_2).

25

Following the general details from Scheme 2, the required bicycle building (2*S*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (6c) was converted to building block-linker construct (8c) as follows:

30 (2*S*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (6c) (354mg, 1.05mmol, 1 eq) was dissolved in a mixture of ethanol (9.6 mL) and water (1.4 mL) containing sodium acetate.trihydrate (218mg, 1.6 mmol, 1.5 eq). 4-[[[(hydrazinocarbonyl)amino] methyl]cyclohexanecarboxylic acid.

trifluoroacetate (346mg, 1.05mmol, 1 eq, Murphy, A. M. *et al*, *J. Am. Chem. Soc.*, 114, 3156-3157, 1992) was added and the mixture refluxed for 2hr. Chloroform (100mL) was added and the organics washed with dilute aqueous hydrochloric acid (0.1 M, 1 x 100 mL). The acidic layer was backwashed with chloroform (3 x 100mL) and the combined organics washed with brine (100 mL), dried (Na₂SO₄) and evaporated under reduced pressure to afford linker construct (8c) as a white solid (660mg). Analytical HPLC indicated one main peak at $R_t = 17.14$ min, HPLC-MS (main UV peak with $R_t = 7.91$ min, 535.3 [M + H]⁺. Crude (8c) was used directly for construct loading.

10

Following the general details from Scheme 2, the required building block-linker construct (8c) was attached to the solid phase providing loaded building block-linker construct (9c) as follows:

15 Building block-linker construct (8c) (1.0mmole), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluoro phosphate (HBTU, 379mg, 1.0mmole), 1-hydroxybenzotriazole.hydrate and (HOBt, 153mg, 1.0mmole) were dissolved in dimethylformamide (5mL) and N-methylmorpholine (NMM, 220μL, 2.0mmole) added. After pre-activation for 5 minutes, free amine gears (250 x 1.2μmole) were added, followed by dimethylformamide (30mL) and left overnight. The spent coupling solution was then added to free amine crowns (25 x 10μmole) and left overnight. Standard washing and analyses indicated loading at >95%.

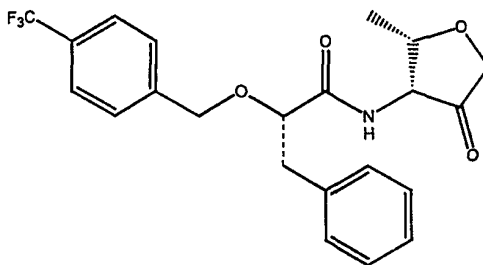
25 Following the general details from Scheme 2, the required loaded building block-linker construct (9c) was elaborated on the solid phase as follows:

2RS-benzyloxy-3-phenylpropionic acid was coupled under standard conditions to loaded building block-linker construct (9c) (following standard removal of Fmoc), then cleaved to provide EXAMPLE 24. The crude example was analysed (see general techniques). HPLC $R_t = 18.34$ mins (> 90 %), HPLC-MS 354.2 [M + H]⁺.

30

The following examples (25 – 34) were prepared as detailed for EXAMPLE 24, coupling with the required reagents to provide the full length molecule.

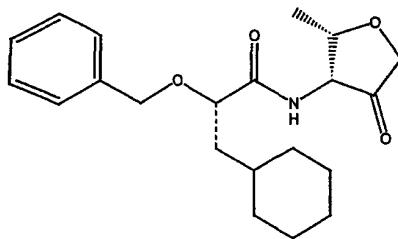
EXAMPLE 25. (2*S*, 3*R*) *N*-(2-Methyl-4-oxo-tetrahydrofuran-3-yl)-3-phenyl-2-(4-trifluoromethylbenzyloxy)-propionamide



HPLC Rt = 20.73 mins (> 90 %), HPLC-MS 422.1 [M + H]⁺, 444.1 [M + Na]⁺.

- 10 The required carboxylic acid, 2*RS*-3-Phenyl-2-(4-trifluoromethylbenzyloxy)-propionic acid was prepared following the general method described for compound (12) as a colourless oil, yield 12.7 mg. HPLC-MS (single main UV peak with Rt = 9.59mins, 325.1 [M + H]⁺).
- 15 ¹H (CDCl₃ at 298K); 2.80-3.17 (2H, PhCH₂, m), 3.90-4.14 (1H, OCH₂CO, m), 4.19-4.37 (1H, CH₂O, d, *J* = 12.57 Hz), 4.45-4.72 (1H, CH₂O, d, *J* = 12.53 Hz), 6.85-7.46 (9H, ArH, m).

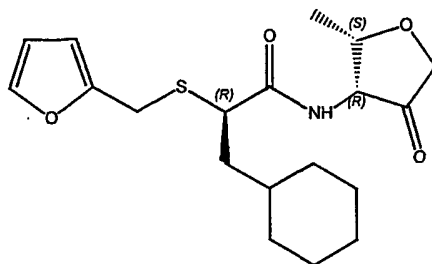
EXAMPLE 26. (2*S*, 3*R*) 2-Benzoyloxy-3-cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



HPLC Rt = 20.73 mins (> 90 %), HPLC-MS 360.2 [M + H]⁺, 741.4 [2M + Na]⁺.

Prepared as detailed for EXAMPLE 1, but using loaded building block-linker construct (9c)

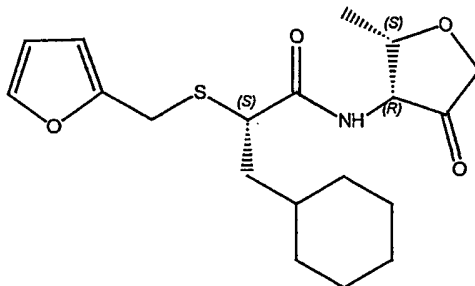
- 5 EXAMPLE 27. (2*S*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



HPLC Rt = 19.71 mins (> 80 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [M + Na]⁺.

- 10 Prepared as detailed for EXAMPLE 3, but using loaded building block-linker construct (9c)

EXAMPLE 28. (2*S*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



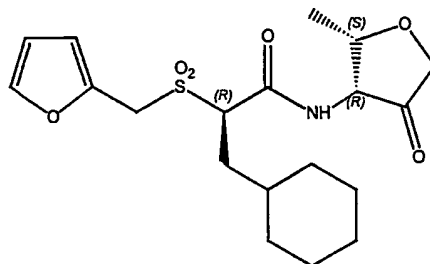
15

HPLC Rt = 19.71 mins (> 80 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [M + Na]⁺.

Prepared as detailed for EXAMPLE 4, but using loaded building block-linker construct (9c)

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EXAMPLE 29. (2*S*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



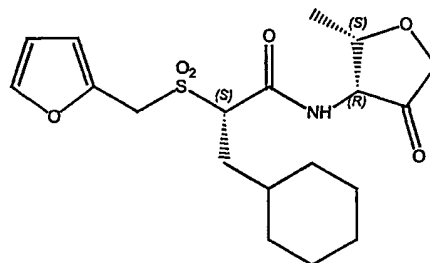
HPLC Rt = 17.63 mins (> 80 %), HPLC-MS 398.1 [M + H]⁺, 817.1 [M + Na]⁺.

5

Prepared as detailed for EXAMPLE 5, but using loaded building block-linker construct (9c)

EXAMPLE 30. (2*S*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide

10

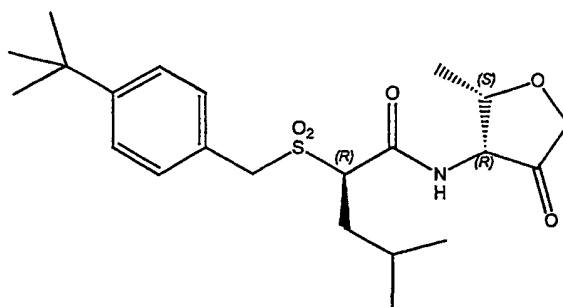


HPLC Rt = 17.93 mins (> 95 %), HPLC-MS 398.1 [M + H]⁺, 817.1 [M + Na]⁺.

Prepared as detailed for EXAMPLE 6, but using loaded building block-linker construct (9c)

15

EXAMPLE 31. (2*S*, 3*R*) 2-(4-*tert*-Butyl-phenylmethanesulfonyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide

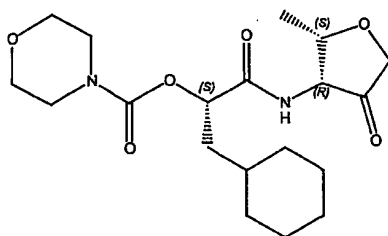


HPLC Rt = 21.13 mins (> 95 %), HPLC-MS 424.2 [M + H]⁺, 869.4 [2M + Na]⁺.

Prepared as detailed for EXAMPLE 7, but using loaded building block-linker construct (9c)

5

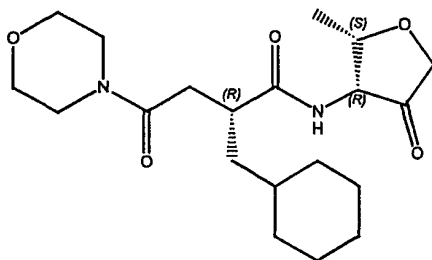
EXAMPLE 32. (2*S*, 3*R*) Morpholine-4-carboxylic acid 2-cyclohexyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester



HPLC-MS 383.4 [M + H]⁺.

10 Prepared as detailed for EXAMPLE 8, but using loaded building block-linker construct (9c)

EXAMPLE 33. (2*S*, 3*R*) 2-Cyclohexylmethyl-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-4-morpholin-4-yl-4-oxo-butyramide

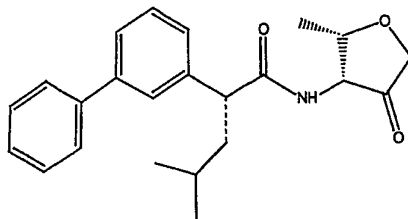


15 HPLC-MS 381.4 [M + H]⁺.

Prepared as detailed for EXAMPLE 9, but using loaded building block-linker construct (9c)

20 EXAMPLE 34. (2*S*, 3*R*) 2-Biphenyl-3-yl-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide

-95-

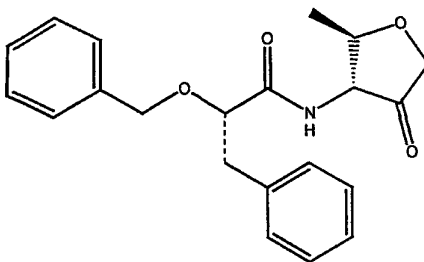


HPLC Rt = 20.66 mins (> 95 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [2M + Na]⁺.

- 5 Prepared as detailed for EXAMPLE 10, but using loaded building block-linker construct (9c)

EXAMPLE 35. (2*R*, 3*R*) 2-Benzyloxy-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-3-phenyl-propionamide

10

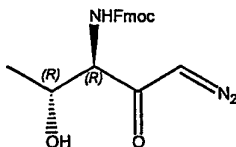


HPLC Rt = 18.35 mins (> 90 %), HPLC-MS 354.2 [M + H]⁺, 376.1 [M + Na]⁺, 729.3 [2M + Na]⁺.

15

Following the general details from Scheme 1, the required bicycle building block (2*R*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9H-fluoren-9-ylmethyl ester (6d) was prepared as follows:

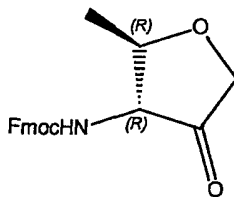
- 20 (1) Preparation of (1*R*, 1'*R*) [3-diazo-1-(1-hydroxyethyl)-2-oxopropyl]carbamic acid 9H-fluoren-9-ylmethyl ester.



A solution of *iso*-butyl chloroformate (0.751 g, 5.5 mmol) in dichloromethane (10 ml) and a solution of 4-methylmorpholine (1.01 g, 10 mmol) in dichloromethane (10 ml) were simultaneously added in four portions to a stirred suspension of Fmoc-D-
5 *allo*-threonine (1.71 g, 5mmol) in dichloromethane (50 ml) at -15 °C over 10 minutes under an atmosphere of nitrogen. Ethereal diazomethane [generated from diazald (5.1 g, ~15mmol) addition in diethyl ether (82 ml) to sodium hydroxide (5.73 g) in water (10 ml)/ethanol (20 ml) at 60°C] was then cautiously added and the resulting yellow solution was stirred at room temperature for 90 minutes. Acetic acid (~2 ml)
10 was cautiously added (until effervescence had ceased), then the mixture was diluted with *tert*-butyl methyl ether (50 ml). The ethereal layer was washed with water (3 x 50 ml), dried (Na₂SO₄) and the solvent removed *in vacuo* to leave (1*R*, 1'*R*) [3-diazo-1-(1-hydroxyethyl)-2-oxopropyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester as a yellow solid (2.43 g) which was used without further purification.

15

(2) Cyclisation to (2*R*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester.



20 A solution of lithium chloride (2.12 g, 50mmol) in 80% acetic acid in water (75 ml) was added to (1*R*, 1'*R*) [3-diazo-1-(1-hydroxyethyl)-2-oxopropyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester (2.43 g, ~5mmol). The yellow oily suspension was stirred for 2 hours whereupon a pale yellow solution formed accompanied by the evolution of a gas. The solvents were removed *in vacuo* then the residue was dissolved in ethyl
25 acetate (30 ml), washed with 10% aqueous sodium carbonate solution (2 x 30 ml) and saturated aqueous sodium chloride solution (30 ml). The combined aqueous layers were extracted with ethyl acetate (2 x 30 ml) then the combined ethyl acetate layers were dried (Na₂SO₄) and the solvents removed *in vacuo* to leave a yellow gum

- (1.59 g). The yellow gum was purified by chromatography over silica gel eluting with a gradient of n-heptane : ethyl acetate 9:1 \rightarrow 7:3. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (2*R*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester as a white crystalline solid (796 mg, 47% from starting acid). TLC (single UV spot, R_f = 0.27, n-heptane : ethyl acetate 2:1), analytical HPLC peak R_t = 18.04mins, HPLC-MS (single UV peak with R_t = 8.47mins, 338.1 $[M + H]^+$, 360.0 $[M + Na]^+$, 361.1 $[M + H + Na]^+$).
- 10 1H ($CDCl_3$ at 298K); 1.51-1.52 (3H, $\underline{CH_3}CHO$, brs), 3.81-3.83 (1H, $\underline{CH_3}CHO$ m), 3.98-4.03 (2H, Fmoc $\underline{CH_2}$, m), 4.14-4.17 (2H, Fmoc H-9 and \underline{NHCHCO} , m), 4.29-4.53 (2H, $\underline{OCH_2CO}$, dd), 5.18-5.19 (1H, \underline{NH} , bs), 7.28-7.32 (2H, ArH, Fmoc H-2 and H-7), 7.34-7.36 (2H, ArH, Fmoc H-3 and H-6), 7.41-7.54 (2H, ArH, Fmoc H-1 and H-8), 7.59-7.80 (2H, ArH, Fmoc H-4 and H-5).
- 15 ^{13}C ($CDCl_3$ at 298K); 18.27 (u, $\underline{CH_3}CHO$), 46.35 (u, Fmoc C-9), 61.91 (u, \underline{NHCHCO}), 66.46 (d, Fmoc $\underline{CH_2}$), 70.03 (d, $\underline{COCH_2}$), 76.60 (u, $\underline{CH_3}CHO$), 119.26 (u, Fmoc C-4 and C-5), 124.21 (u, Fmoc C-1 and C-8), 126.35 (u, Fmoc C-2 and C-7), 127.03 (u, Fmoc C-3 and C-6), 140.57 (q, Fmoc C-4' and C-5'), 142.81/142.86 (q, Fmoc C-1' and C-8'), 155.27 (\underline{OCON}), 211.05 ($\underline{COCH_2}$).
- 20 Following the general details from Scheme 2, the required bicycle building (2*R*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (**6d**) was converted to building block-linker construct (**8d**) as follows:
- 25 (2*R*, 3*R*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (**6d**) (354mg, 1.05mmol, 1 eq) was dissolved in a mixture of ethanol (9.6 mL) and water (1.4 mL) containing sodium acetate.trihydrate (218mg, 1.6 mmol, 1.5 eq). 4-[[[(hydrazinocarbonyl)amino] methyl]cyclohexanecarboxylic acid. trifluoroacetate (346mg, 1.05mmol, 1 eq, Murphy, A. M. *et al*, *J. Am. Chem. Soc.*,
- 30 114, 3156-3157, 1992) was added and the mixture refluxed for 2hr. Chloroform (100mL) was added and the organics washed with dilute aqueous hydrochloric acid (0.1 M, 1 x 100 mL). The acidic layer was backwashed with chloroform (3 x 100mL)

and the combined organics washed with brine (100 mL), dried (Na_2SO_4) and evaporated under reduced pressure to afford linker construct (8d) as a white solid (620mg). Analytical HPLC indicated one main peak at $R_t = 17.20$ min, HPLC-MS (main UV peak with $R_t = 7.91$ min, $535.3 [\text{M} + \text{H}]^+$). Crude (8d) was used directly for
5 construct loading.

Following the general details from Scheme 2, the required building block-linker construct (8d) was attached to the solid phase providing loaded building block-linker construct (9d) as follows:

10

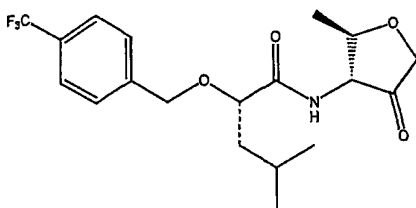
Building block-linker construct (8d) (1.0mmole), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluoro phosphate (HBTU, 379mg, 1.0mmole), 1-hydroxybenzotriazole.hydrate and (HOBT, 153mg, 1.0mmole) were dissolved in dimethylformamide (5mL) and N-methylmorpholine (NMM, 220 μ L, 2.0mmole)
15 added. After pre-activation for 5 minutes, free amine gears (250 x 1.2 μ mole) were added, followed by dimethylformamide (30mL) and left overnight. The spent coupling solution was then added to free amine crowns (25 x 10 μ mole) and left overnight. Standard washing and analyses indicated loading at >95%.

20 Following the general details from Scheme 2, the required loaded building block-linker construct (9d) was elaborated on the solid phase as follows:

2*RS*-benzyloxy-3-phenylpropionic acid was coupled under standard conditions to loaded building block-linker construct (9d) (following standard removal of Fmoc),
25 then cleaved to provide EXAMPLE 35. The crude example was analysed (see general techniques). HPLC $R_t = 18.35$ mins (> 90 %), HPLC-MS 354.2 $[\text{M} + \text{H}]^+$, 376.1 $[\text{M} + \text{Na}]^+$, 729.3 $[2\text{M} + \text{Na}]^+$.

The following examples (36 – 47) were prepared as detailed for EXAMPLE 35,
30 coupling with the required reagents to provide the full length molecule.

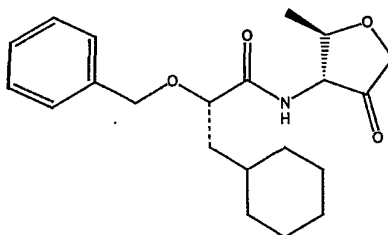
EXAMPLE 36. (2*R*, 3*R*) 4-Methyl-2-(4-trifluoromethylbenzyloxy)-pentanoic acid
(2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



5 HPLC $R_t = 20.69$ mins ($> 90\%$), HPLC-MS 388.2 $[M + H]^+$, 797.2 $[2M + Na]^+$.

Prepared as detailed for EXAMPLE 12, but using loaded building block-linker construct (9d)

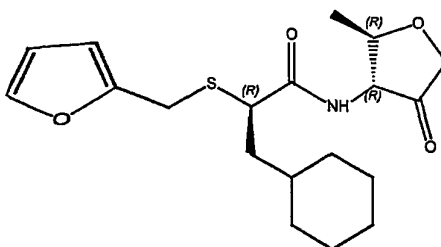
10 EXAMPLE 37. (2*R*, 3*R*) 2-Benzyloxy-3-cyclohexyl-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



15 HPLC $R_t = 20.70$ mins ($> 90\%$), HPLC-MS 360.2 $[M + H]^+$, 741.4 $[2M + Na]^+$.

Prepared as detailed for EXAMPLE 1, but using loaded building block-linker construct (9d)

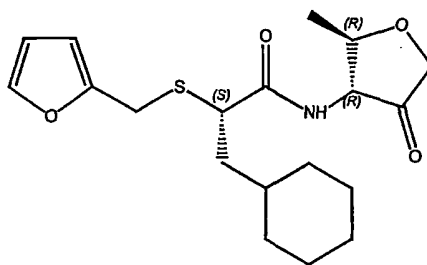
20 EXAMPLE 38. (2*R*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



HPLC Rt = 19.71 mins (> 80 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [M + Na]⁺.

- 5 Prepared as detailed for EXAMPLE 3, but using loaded building block-linker construct (9d)

EXAMPLE 39. (2*R*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethylsulfanyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide

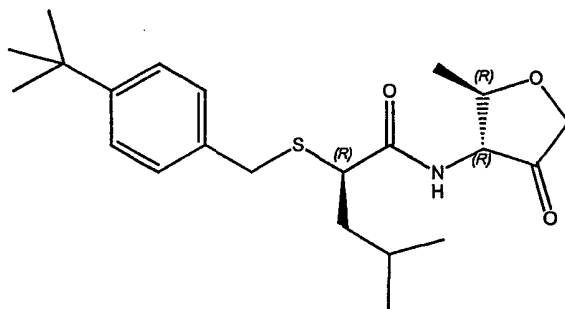


10

HPLC Rt = 19.71 mins (> 80 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [M + Na]⁺.

- Prepared as detailed for EXAMPLE 4, but using loaded building block-linker
15 construct (9d)

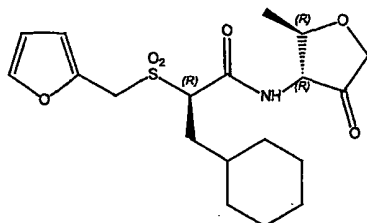
EXAMPLE 40. (2*R*, 3*R*) 2-(4-*tert*-Butyl-benzylsulfanyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



- 20 HPLC Rt = 23.23 mins (> 95 %), HPLC-MS 392.2 [M + H]⁺, 805.4 [2M + Na]⁺.

Prepared as detailed for EXAMPLE 7, but using loaded building block-linker construct (9d) and no oxidation of the intermediate thioether.

EXAMPLE 41. (2*R*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



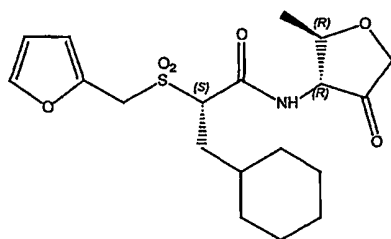
5

HPLC Rt = 17.67 mins (> 80 %), HPLC-MS 398.1 [M + H]⁺, 817.1 [M + Na]⁺.

Prepared as detailed for EXAMPLE 5, but using loaded building block-linker construct (9d)

10

EXAMPLE 42. (2*R*, 3*R*) 3-Cyclohexyl-2-(furan-2-ylmethanesulfonyl)-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-propionamide



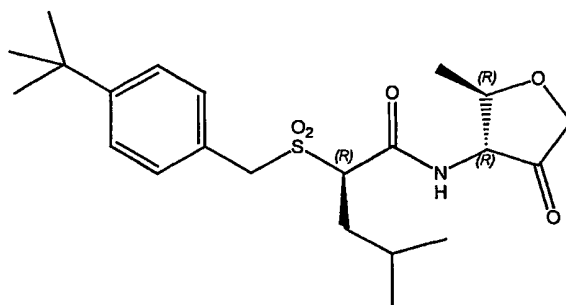
15

HPLC Rt = 17.93 mins (> 95 %), HPLC-MS 398.1 [M + H]⁺, 817.1 [M + Na]⁺.

Prepared as detailed for EXAMPLE 6, but using loaded building block-linker construct (9d)

EXAMPLE 43. (2*R*, 3*R*) 2-(4-*tert*-Butyl-phenylmethanesulfonyl)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide

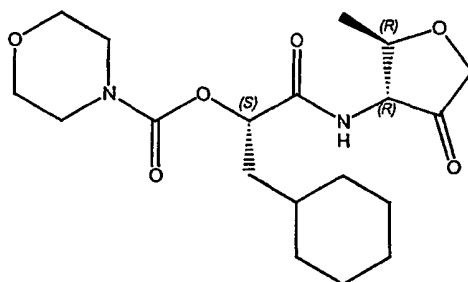
20



HPLC Rt = 21.19 mins (> 95 %), HPLC-MS 424.2 [M + H]⁺, 869.4 [2M + Na]⁺.

Prepared as detailed for EXAMPLE 7, but using loaded building block-linker
5 construct (9d)

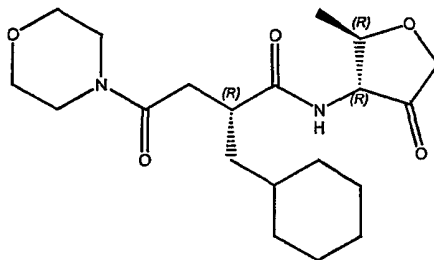
EXAMPLE 44. (2*R*, 3*R*) Morpholine-4-carboxylic acid 2-cyclohexyl-1-(2-methyl-4-oxo-tetrahydro-furan-3-ylcarbamoyl)-ethyl ester



10 HPLC-MS 383.4 [M + H]⁺.

Prepared as detailed for EXAMPLE 8, but using loaded building block-linker
construct (9d)

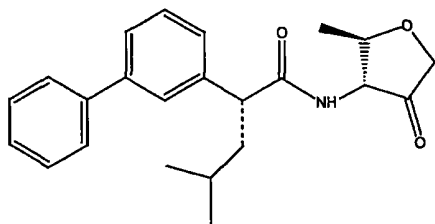
15 EXAMPLE 45. (2*R*, 3*R*) 2-Cyclohexylmethyl-*N*-(2-methyl-4-oxo-tetrahydrofuran-3-yl)-4-morpholin-4-yl-4-oxo-butyramide



20 HPLC-MS 381.4 [M + H]⁺.

Prepared as detailed for EXAMPLE 9, but using loaded building block-linker
construct (9d)

EXAMPLE 46. (2*R*, 3*R*) 2-Biphenyl-3-yl-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



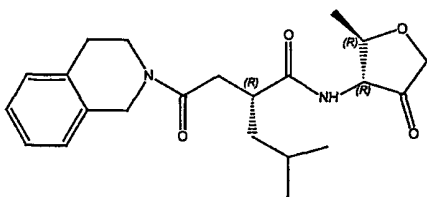
5

HPLC Rt = 20.62 mins (> 95 %), HPLC-MS 366.1 [M + H]⁺, 753.2 [2M + Na]⁺.

Prepared as detailed for EXAMPLE 10, but using loaded building block-linker construct (9d)

10

EXAMPLE 47. (2*R*, 3*S*) 2-[2-(3,4-Dihydro-1H-isoquinolin-2-yl)-2-oxo-ethyl]-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



15 HPLC Rt = 16.04 & 16.54mins (> 80%), HPLC-MS 387.2 [M + H]⁺, 795.4 [2M + Na]⁺.

Prepared as detailed for EXAMPLE 23, but using loaded building block-linker construct (9d)

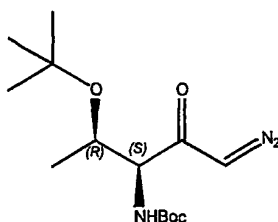
20

Solution Phase Syntheses

EXAMPLES (48-51) were prepared by standard solution phase chemistries, coupling with the required reagents to provide the full length molecule. The core building

block (4*S*, 5*R*) 4-amino-5-methyl-dihydro-furan-3-one hydrochloride (Compound (31), Schemes 9 and 10) was prepared as follows:

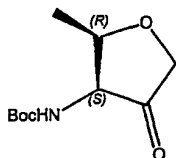
- (1) Preparation of (1*S*, 1'*R*) [1-(1-*tert*-butoxyethyl)-3-diazo-2-oxopropyl]carbamic acid *tert*-butyl ester



- A solution of *iso*-butyl chloroformate (0.998 ml, 7.7 mmol) in dichloromethane (14 ml) and a solution of 4-methylmorpholine (1.54 ml, 14 mmol) in dichloromethane (14 ml) were simultaneously added to a stirred suspension of Boc-*O*-(*tert*-butyl)-L-threonine (1.93 g, 7mmol) in dichloromethane (70 ml) at -15 °C over 10 minutes under an atmosphere of argon. Ethereal diazomethane [generated from diazald (7.14 g, ~21mmol) addition in diethyl ether (115 ml) to sodium hydroxide (8.02 g) in water (11 ml)/ethanol (31 ml) at 60°C] was then cautiously added and the resulting yellow solution was stirred at room temperature for 90 minutes. Acetic acid (~2 ml) was cautiously added (until effervescence had ceased) then the mixture was diluted with *tert*-butyl methyl ether (50 ml). The ethereal layer was washed with water (3 x 50 ml), dried (Na₂SO₄) and the solvent removed *in vacuo* to leave (1*S*, 1'*R*) [1-(1-*tert*-butoxyethyl)-3-diazo-2-oxopropyl]carbamic acid *tert*-butyl ester as a yellow solid (2.81 g) which was used without further purification.

20

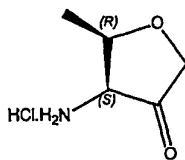
- (2) Cyclisation to (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid *tert*-butyl ester



- A solution of lithium chloride (2.97 g, 70mmol) in 80% acetic acid in water (105 ml) was added to (1*S*, 1'*R*) [1-(1-*tert*-butoxyethyl)-3-diazo-2-oxopropyl]carbamic acid *tert*-butyl ester (2.81 g, ~7mmol). The yellow oily suspension was stirred for 2 hours whereupon a pale yellow solution formed accompanied by the evolution of a gas.
- 5 The solvents were removed *in vacuo* then the residue was dissolved in ethyl acetate (42 ml), washed with 10% aqueous sodium carbonate solution (2 x 42 ml) and saturated aqueous sodium chloride solution (42 ml). The combined aqueous layers were extracted with ethyl acetate (2 x 42 ml) then the combined ethyl acetate layers were dried (Na₂SO₄) and the solvents removed *in vacuo* to leave a yellow gum (1.1
- 10 g). The yellow gum was purified by chromatography over silica gel eluting with n-heptane : ethyl acetate 3:1. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid *tert*-butyl ester as a white crystalline solid (393 mg, 26% from starting acid). TLC (single UV spot, R_f = 0.31, n-heptane : ethyl acetate 2:1), HPLC-MS (single
- 15 UV peak with R_t = 6.448mins, 238.1 [M + Na]⁺, 453.3 [2M + Na]⁺).

- ¹H (CDCl₃ at 298K); 1.47 (9H, C(CH₃)₃, s), 1.53 (3H, CH₃CHO, d, *J* = 6.02 Hz), 3.68-3.81 (1H, CH₃CHO, m), 3.90-4.05 (2H, COCH₂O and NHCHCO, m), 4.20-4.31 (1H, OCH₂CO, d, *J* = 17.36 Hz), 4.84-4.97 (1H, NH, brs).
- 20 ¹³C (CDCl₃ at 298K); 19.50 (u, CH₃CHO), 28.61 (u, C(CH₃)₃), 62.91 (u, Cα), 71.23 (d, COCH₂), 78.06 (u, CH₃CHO), 81.03 (q, C(CH₃)₃), 155.73 (q, OCON), 212.85(q, COCH₂).

(3) Preparation of (4*S*, 5*R*) 4-amino-5-methyl-dihydrofuran-3-one hydrochloride.

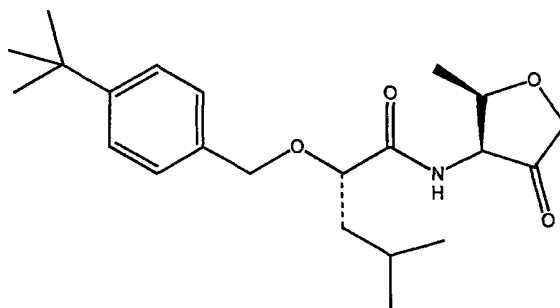


25

HCl in dioxane (4.0M, 9ml) was added drop-wise with stirring over 1 minute to (2*R*, 3*S*) (2-methyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid *tert*-butyl ester (0.372 g, 1.73mmol). The mixture was stirred for 20 minutes then the solvents evaporated in

vacuo to leave a residue which was azeotroped with toluene (2 x 20 ml) to leave (4*S*, 5*R*) 4-amino-5-methyl-dihydro-furan-3-one hydrochloride as a white solid (266 mg, 100%). HPLC-MS, $R_t = 0.419$ mins, 116.1 $[M+H]^+$.

- 5 EXAMPLE 48. (2*R*, 3*S*) 2-(4-*tert*-Butylbenzyloxy)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



HPLC $R_t = 21.429$ mins, HPLC-MS 376.2 $[M + H]^+$, 773.4 $[2M + Na]^+$.

- 10 The required ether carboxylic acid building block 2*RS*-(4-*tert*-butyl-benzyloxy)-4-methyl-pentanoic acid (compound (33), Scheme 10) was prepared from (*S*)-2-hydroxyisocaproic acid (32) as follows:

- (1) Preparation of 2*RS*-(4-*tert*-butylbenzyloxy)-4-methyl-pentanoic acid (compound
15 (33), Scheme 10)

- A solution of (*S*)-2-hydroxyisocaproic acid (compound (32) 0.66 g, 5mmol) in dichloromethane (10 ml) was added to a suspension of sodium hydride (0.44g of 60% dispersion in oil, 11mmol) in dimethylformamide (20 ml) at 0°C. The mixture
20 was stirred at 0°C for 0.5 hours then a solution of 4-*tert*-butylbenzyl bromide (1.42 g, 6.25mmol) in dichloromethane (5 ml) was added drop-wise. The suspension was stirred for 2 hours then 1M hydrochloric acid (40 ml) was added and the product extracted into dichloromethane (100 ml) then washed with saturated aqueous sodium chloride solution (2 x 50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo* to
25 leave a residue. The residue was purified by chromatography over silica gel eluting with methanol : dichloromethane 1:100. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 2*RS*-(4-*tert*-butylbenzyloxy)-4-methyl-

pentanoic acid as a yellow oil (79 mg, 6%). Analytical HPLC peak $R_t = 21.310$ mins, HPLC-MS (single peak with $R_t = 10.424$ mins, $301.1 [M+Na]^+$, $579.2 [2M + Na]^+$).

1H ($CDCl_3$ at 298K); 0.71 and 0.88 (6H, $CH_2CH(CH_3)_2$, both d, $J = 6.6$ Hz), 1.23
 5 (9H, $C(CH_3)_3$, s), 1.42-1.54 ($1HCH_2CH(CH_3)_2$, m), 1.63-1.74 ($1HCH_2CH(CH_3)_2$, m), 1.75-1.87 (1H, $CH_2CH(CH_3)_2$, m), 3.88-3.97 (1H, $OCHCOOH$, m), 4.28-4.35 (1H, $ArCH_2O$, d, $J = 11.26$ Hz), 4.55-4.67 (1H, $ArCH_2O$, d, $J = 11.25$ Hz), 7.20-7.33 (4H, ArH, m).

^{13}C ($CDCl_3$ at 298K); 23.61 (u, $CH_2CH(CH_3)_2$), 24.83 (u, $CH_2CH(CH_3)_2$), 31.51 (u, $C(CH_3)_3$), 42.14 (d, $CH_2CH(CH_3)_2$), 72.68 (d, $ArCH_2O$), 76.54 (u, $OCHCOOH$),
 10 125.75/125.84 (u, aromatic CH), 128.29/128.48 (u, aromatic CH), 134.71 (q, Ar C), 151.36 (q, Ar C), 178.41 (q, CO).

Carboxylic acid building (33) and (4*S*, 5*R*) 4-amino-5-methyl-dihydro-furan-3-one
 15 hydrochloride (31) were coupled as follows:

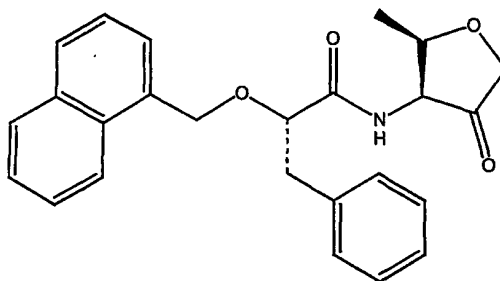
iso-Butyl chloroformate (20.09mg, 0.1471 mmol) in dichloromethane (2ml) and *N*-methyl morpholine (29.75mg, 0.294 mmol) in dichloromethane (2ml) were simultaneously added were simultaneously added to a stirred solution of (31) (37.22
 20 mg, 0.1337mmol) in dichloromethane (2 ml) at $-15^\circ C$ under argon over 5 minutes. The mixture was stirred at $-15^\circ C$ for 15 minutes. A freshly prepared solution of (33) (20.27 mg, 0.1337mmol) in dichloromethane (2 ml) and 4-methylmorpholine (13.52 mg, 0.1337 mmol) was then added dropwise. The mixture was then stirred at ambient temperature for 16 hours. The solvent was removed *in vacuo* to give a residue which
 25 was purified over silica gel eluting with a gradient of n-heptane : ethyl acetate 3:1 to 2:1. Desired fractions were combined and reduced *in vacuo* to leave a white foam (37.10mg, 73.9% from starting acid). TLC (single UV spot, $R_f = 0.85$, 10% MeOH in DCM), analytical HPLC single peak with $R_t = 21.429$ mins, HPLC-MS (single UV peak with $R_t = 10.829$ mins, $376.2 [M + H]^+$, $773.4 [2M + Na]^+$).

30

1H ($CDCl_3$ at 298K); 0.75-0.90 (6H, $(CH_3)_2CH_2$, m), 1.25 (9H, $C(CH_3)_3$, s), 1.34-1.41 (3H, $OCHCH_3$, d, $J = 6.03$ Hz), 1.45-1.63 (2H, $CH_2CH(CH_3)_2$, m), 1.71-1.87 (1H, $CH_2CH(CH_3)_2$, m), 3.71-3.80 (1H, $OCHCH_3$, m), 3.83-4.04 (3H, $OCHCO$,

NHCHCO and COCH₂O, m), 4.12-4.21 (1H, COCH₂O, m), 4.47 (2H, ArCH₂, s), 6.77 (1H, NH, s), 7.18-7.36 (4H, ArH, m).

EXAMPLE 49. (2*R*, 3*S*) *N*-(2-Methyl-4-oxo-tetrahydrofuran-3-yl)-2-(naphthalen-1-ylmethoxy)-3-phenyl-propionamide



Analytical HPLC *R*_t = 20.50mins (major isomer) and 20.64mins (minor isomer) and HPLC-MS (single major UV peak with *R*_t = 9.746mins, 404.2 [M + H]⁺, 426.2 [M + Na]⁺, 829.4 [2M + Na]⁺).

The required ether carboxylic acid building block 2*RS*-(Naphthalen-1-ylmethoxy)-3-phenylpropionic acid was prepared as follows:

15 Sodium hydride (265 mg of 60% dispersion in oil, 6.6mmol) was added in two portions to a stirred mixture of (*S*)-2-hydroxy-3-phenylpropionic acid (0.5 g, 3.0mmol), dimethylformamide (5 ml) and dichloromethane (5 ml) at 0°C over 5 minutes. The mixture was stirred at 0°C for 5 minutes then at ambient temperature for 30 minutes. 1-(Chloromethyl)naphthalene (95%, 0.57 ml, 3.6mmol) was added
20 then the mixture stirred for 5 hours before adding potassium iodide (50 mg, 0.3mmol). The mixture was stirred for 20 hours then heated at 55°C for 1 hour then allowed to cool to ambient temperature and poured into water (15 ml). A saturated aqueous sodium chloride solution (5 ml) was added then the mixture was extracted with dichloromethane (5 ml then 10 ml) that was discarded. The aqueous layer was
25 acidified using 1M hydrochloric acid (10ml) then extracted with dichloromethane (2 x 10 ml). The dichloromethane layer was dried (MgSO₄) and the solvent removed *in vacuo*. The residue (0.48 g) was dissolved in dimethylformamide (6 ml) then cooled

to 0°C before adding sodium hydride (150 mg of 60% dispersion in oil, 3.75mmol). The mixture was stirred for 30 minutes then polymer bound isocyanate (300 mg, 2mmol Ng^{-1}) added. The mixture was stirred for 2 hours at ambient temperature then poured into water (15 ml). 1M Hydrochloric acid (10 ml) was added then the product
5 extracted into dichloromethane (2 x 10 ml), dried (MgSO_4) and the solvent removed *in vacuo*. The residue was dissolved in aqueous sodium hydroxide solution (1M, 10 ml) then extracted with dichloromethane (2 x 10 ml) that was discarded. Crushed ice was added to the aqueous layer followed by 1M hydrochloric acid (12 ml). The product was extracted into dichloromethane (2 x 10 ml) then dried (Na_2SO_4) and the
10 solvent removed *in vacuo*. The residue was purified by flash chromatography over silica gel eluting with a gradient of methanol : dichloromethane 0:1 \rightarrow 1:20. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 2*RS*-(Naphthalen-1-ylmethoxy)-3-phenylpropionic acid (40 mg) as a colourless oil. HPLC-MS (single main UV peak with $R_t = 9.22\text{mins}$, $307.2 [\text{M} + \text{H}]^+$, $329.2 [\text{M} + \text{Na}]^+$).
15 $\text{Na}]^+$).

δH (CDCl_3 at 298K); 2.82-3.18 (2H, PhCH_2 , m), 4.00-4.25 (1H, OCHCO , m), 4.63-4.81 (1H, CH_2O , d, $J = 11.55\text{ Hz}$), 4.92-5.10 (1H, CH_2O , d, $J = 11.52\text{ Hz}$), 6.90-7.86 (12H, ArH, m), 8.78 (1H, OH, brs).

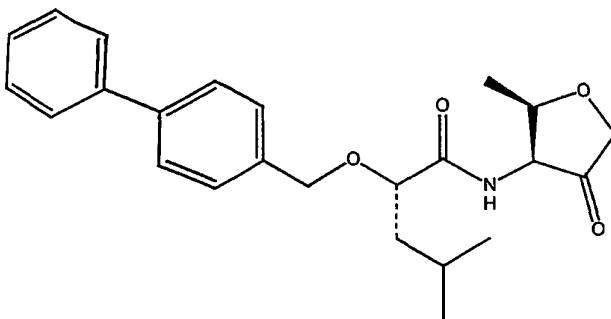
20 2*RS*-(Naphthalen-1-ylmethoxy)-3-phenylpropionic acid and (4*S*, 5*R*) 4-amino-5-methyl-dihydro-furan-3-one hydrochloride (31) were coupled as follows:

Solutions of isobutyl chloroformate (18.6 μl , 0.14mmol) in dichloromethane (0.67 ml) and 4-methylmorpholine (31.6 μl , 0.29mmol) in dichloromethane (0.67 ml) were
25 added simultaneously at -15°C over 10 minutes drop-wise to a stirred solution of 2*RS*-(Naphthalen-1-ylmethoxy)-3-phenylpropionic acid (40 mg, 0.13mmol) in dichloromethane (0.5 ml) under argon. The solution was stirred for 15 minutes then (4*S*, 5*R*) 4-amino-5-methyldihydrofuran-3-one hydrochloride (20 mg, 0.13mmol)
30 followed by a solution of 4-methylmorpholine (14.4 μl , 0.13mmol) in dichloromethane (0.3 ml) were added. The mixture was allowed to warm to 0°C over 3 hours, stirred at ambient temperature for 20 hours then the solvents were removed *in vacuo*. The residue was purified by flash chromatography over silica gel eluting

with a gradient of ethyl acetate : heptane 1:3 \rightarrow 7:13. Appropriate fractions were combined and the solvents removed *in vacuo* to leave (2*R*, 3*S*) *N*-(2-Methyl-4-oxo-tetrahydrofuran-3-yl)-2-(naphthalen-1-ylmethoxy)-3-phenyl-propionamide as a white solid (28.5 mg, 3.5:1 mixture of diastereoisomers, 70%). TLC (two spots, R_f = 0.45
 5 and 0.42, ethyl acetate : heptane 7:13).

^1H (CDCl_3 at 298K); Major isomer 1.4 (3H, CH_3CH , d, J = 6.0Hz), 2.90 (1H, PhCH_2 , dd, J = 14.1, 8.0Hz), 3.15 (1H, PhCH_2 , dd, J = 14.1, 3.6Hz), 3.32 and 3.51 (both 1H, CH_3CHCHN , m), 3.85 (1H, OCH_2CO , d, J = 17.1Hz), 4.08 (1H, OCH_2CO ,
 10 d, J = 17.1Hz), 4.19 (1H, OCHCO , m), 4.6 (1H, $\text{OCH}_2\text{Naphthyl}$, d, J = 11.8Hz), 4.90 (1H, $\text{OCH}_2\text{Naphthyl}$, d, J = 11.8Hz), 6.55 (1H, NH , d, J = 6Hz), 7.18-7.93 (12H, aromatic); Minor isomer 1.50 (3H, CH_3CH , d, J = 6.0Hz), 2.94 (1H, PhCH_2 , m), 3.15 (1H, PhCH_2 , m), 3.52 and 3.89 (both 1H, CH_3CHCHN , m), 4.02-4.22 (3H, OCH_2CO and OCHCO , m), 4.6 (2H, $\text{OCH}_2\text{Naphthyl}$, s), 6.74 (1H, NH , d, J = 6Hz), 7.18-7.93
 15 (12H, aromatic).

EXAMPLE 50. (2*R*, 3*S*) 2-(Biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide



20 HPLC R_t = 20.178mins (> 95 %), HPLC-MS 396.2 $[\text{M} + \text{H}]^+$, 813.4 $[2\text{M} + \text{Na}]^+$.

The required ether carboxylic acid building block 2*RS* 2-(Biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid was prepared as follows:

25 (1) Preparation of 2*RS*-(Biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid methyl ester

Sodium hydride (98.4 mg of 60% dispersion in oil, 2.46mmol) was added in two portions to a stirred solution of 2*S*-hydroxy-4-methyl-pentanoic acid methyl ester (300 mg, 2.05mmol) in tetrahydrofuran (2.5 ml) at 0°C under an atmosphere of nitrogen over 5 minutes. The mixture was stirred at ambient temperature for 10 minutes then a solution of 4-phenylbenzylchloride (830mg, 4.1mmol) followed by potassium iodide (25mg) was added. The mixture was stirred for 20 hours then saturated aqueous ammonium chloride solution (40 ml) was added. The product was extracted into ethyl acetate (3 x 30 ml) then the combined ethyl acetate layers were washed with aqueous saturated sodium chloride solution, dried (Na₂SO₄) and the solvents removed *in vacuo*. The residue (1.2 g) was purified by flash chromatography over silica gel eluting with ethyl acetate : heptane 1:20 → 1 : 9. Appropriate fractions were combined and the solvents removed *in vacuo* to leave 3-cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid methyl ester (110 mg, 17.1%) as a colourless oil. TLC (single spot, R_f = 0.2, heptane : ethyl acetate 9:1), HPLC-MS main UV peak with 313.1 [M + H]⁺.

(2) Preparation of 2*RS*-(Biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid.

A solution of lithium hydroxide monohydrate (0.0443 g, 1.056mmol) in water (3 ml) was added drop-wise over 1 minute to a solution of 2-(biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid methyl ester (0.11 g, 0.3521mmol) in tetrahydrofuran : methanol 2:1 (15 ml) at 0°C. The mixture was stirred at ambient temperature for 16 hours then poured into saturated aqueous sodium chloride solution (10 ml). The aqueous layer was extracted with diethyl ether (2 x 10 ml) which was discarded. The aqueous layer was acidified with 1M hydrochloric acid to pH (1~2), then the product was extracted into dichloromethane (3 x 10 ml). The combined dichloromethane layers were washed with water (2 x 10 ml), saturated aqueous sodium chloride solution (2 x 10 ml) then dried (Na₂SO₄) and the solvents removed *in vacuo* to leave 2-(biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid as a colourless oil (0.048 g, 45.69%). TLC (single UV spot, R_f = 0.2, methanol : dichloromethane 0.5:9.5), HPLC-MS (297.1 [M-1]⁺, 321.1 [M + Na]⁺, 619.3 [2M + Na]⁺).

^1H (CDCl_3 at 298K); 0.71-0.94 (6H, $(\text{CH}_3)_2\text{CH}$, two d, $J_{1a} = 6.57$, $J_{1b} = 6.66$ Hz, $J_2 = 29.87$ Hz), 1.19 (1H, OH, bs), 1.45-1.76 (2H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, m), 1.77-1.92 (1H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, m), 2.93-4.05 (1H, CHCOOH , m), 4.37-4.48 (1H, CH_2Ar , d, $J = 11.43$ Hz), 4.64-4.75 (1H, CH_2Ar , d, $J = 11.42$ Hz), 7.23-7.60 (9H, ArH, m).

5 ^{13}C (CDCl_3 at 298K); 23.17 (u, $(\text{CH}_3)_2\text{CH}$), 24.81/26.13 (u, $(\text{CH}_3)_2\text{CH}$), 43.30 (d, $(\text{CH}_3)_2\text{CHCH}_2$), 74.06 (d, ArCH_2), 78.02 (u, OCHCOOH), 128.77/128.91/129.03.43 (u, Ar), 130.31/130.44 (u, Ar), 137.71 (q, Ar), 142.42/142.71 (q, Ar), 179.79 (q, OCO).

10

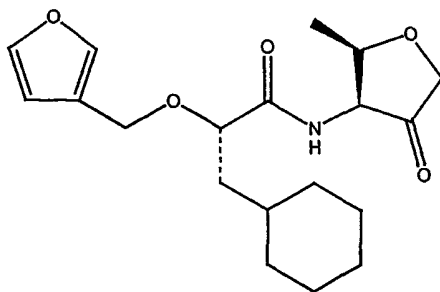
2*RS*-2-(Biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid and (4*S*, 5*R*) 4-amino-5-methyl-dihydro-furan-3-one hydrochloride (**31**) were coupled as follows:

15 *iso*-Butyl chloroformate (15.61mg, 0.1143 mmol) in dichloromethane (2ml) and *N*-methyl morpholine (23.12mg, 0.2286 mmol) in dichloromethane (2ml) were simultaneously added to a stirred solution of 2*RS*-2-(biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid (31mg, 0.104mmol) in dichloromethane (2 ml) at -15°C under argon over 5 minutes. The mixture was stirred at -15°C for 15 minutes. A freshly prepared solution of (**31**) (15.75 mg, 20 0.104mmol) in dichloromethane (2 ml) and 4-methylmorpholine (11.56 mg, 0.1143 mmol) was then added dropwise. The mixture was then stirred at ambient temperature for 16 hours. The solvent was removed *in vacuo* to give a residue which was purified over silica gel eluting with a gradient of *n*-heptane : ethyl acetate 3:1. Desired fractions were combined and reduced *in vacuo* to leave (2*R*, 3*S*) 2- 25 (Biphenyl-4-ylmethoxy)-4-methyl-pentanoic acid (2-methyl-4-oxo-tetrahydrofuran-3-yl)-amide as an off-white gum (34mg, 82.7% from starting acid). TLC (single UV spot, $R_f = 0.85$, 10% MeOH in DCM).

30 ^1H (CDCl_3 at 298K); 0.72-0.93 (6H, $(\text{CH}_3)_2\text{CH}_2$, m), 1.39 (3H, OCHCH_3 , d, $J = 6.04$ Hz), 1.45-1.69 (2H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, m), 1.73-1.89 (1H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, m), 3.70-3.81 (1H, OCHCH_3 , m), 3.84-4.08 (3H, OCHCONH , COCH_2O and NHCHCOCH_2 , m), 4.10-4.20 (1H, COCH_2O , d, $J = 17.35$ Hz), 4.40-4.67 (2H, ArCH_2O , m), 6.71 (1H, NH, bs), 7.60-7.24 (9H, ArH, m).

^1H NMR (CDCl_3 at 298K); 17.19/17.27 (u, CH_3 , furanone), 19.70/19.89/19.94 (u, $\text{CH}_2\text{CH}(\text{CH}_3)_2$), 21.38/21.43/22.74/22.79 (u, $\text{CH}_2\text{CH}(\text{CH}_3)_2$), 40.42/40.59/40.80 (d, $\text{OCHCH}_2\text{CH}(\text{CH}_3)_2$), 59.29 (u, NHCHCOCH_2), 69.17/69.24 (d, ArCH_2O), 70.93/71.05/71.28 (d, COCH_2), 74.68 (u, $\text{C}\beta$), 77.11/77.22 (u, OCHCONH),
 5 125.22/125.51/125.57/125.60/125.63/126.68/126.97/127.18 (u, Ar CH),
 133.81/133.98/134.11 (q, Ar C), 138.69/138.75/139.40/139.43 (q, Ar C),
 171.99/172.23 (q, CON), 209.36/209.98/210.21/172.23 (q, COCH_2).

10 EXAMPLE 51. (2*R*, 3*S*) 3-Cyclohexyl-2-(furan-3-ylmethoxy)-*N*-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide



HPLC R_t = 17.858mins (> 90%), HPLC-MS 350.2 $[\text{M} + \text{H}]^+$, 372.2 $[\text{M} + \text{Na}]^+$, 721.3 $[2\text{M} + \text{Na}]^+$.

The required ether carboxylic acid building block 2*RS* 3-Cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid was prepared as follows:

(1) Preparation of 3-cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid methyl ester.

Sodium hydride (204 mg of 60% dispersion in oil, 5.1mmol) was added in two portions to a stirred solution of 3-cyclohexyl-2*S*-hydroxypropionic acid methyl ester (18) (794 mg, 4.27mmol) in tetrahydrofuran (5 ml) at 0°C under an atmosphere of nitrogen over 5 minutes. The mixture was stirred at ambient temperature for 10 minutes then a solution of 3-bromomethylfuran (2.75 g, 17.08mmol) in tetrahydrofuran (1 ml) was added. The mixture was stirred for 20 hours then saturated aqueous ammonium chloride solution (40 ml) was added. The product was

extracted into ethyl acetate (3 x 30 ml) then the combined ethyl acetate layers were washed with aqueous saturated sodium chloride solution, dried (Na_2SO_4) and the solvents removed *in vacuo*. The residue (2.0 g) was purified by flash chromatography over silica gel eluting with ethyl acetate : heptane 1:4. Appropriate
5 fractions were combined and the solvents removed *in vacuo* to leave 3-cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid methyl ester (64 mg, 6%) as a colourless oil. TLC (single spot, $R_f = 0.2$, heptane : ethyl acetate 9:1), HPLC-MS (main UV peak with $R_t = 10.701$ mins, 289.1 $[\text{M} + \text{Na}]^+$, 555.3 $[2\text{M} + \text{Na}]^+$).

10 (2) Preparation of 3-cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid.

A solution of lithium hydroxide monohydrate (0.03025 g, 0.721 mmol) in water (3 ml) was added drop-wise over 1 minute to a solution of 3-cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid methyl ester (0.064 g, 0.240 mmol) in tetrahydrofuran :
15 methanol 2:1 (15 ml) at 0°C . The mixture was stirred at ambient temperature for 16 hours then poured into saturated aqueous sodium chloride solution (10 ml). The aqueous layer was extracted with diethyl ether (2 x 10 ml) which was discarded. The aqueous layer was acidified with 1M hydrochloric acid to pH (1~2), then the product was extracted into dichloromethane (3 x 10 ml). The combined dichloromethane
20 layers were washed with water (2 x 10 ml), saturated aqueous sodium chloride solution (2 x 10 ml) then dried (Na_2SO_4) and the solvents removed *in vacuo* to leave Preparation of 3-cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid as a light yellow oil (0.012 g, 19.80%). TLC (single UV spot, $R_f = 0.35$, methanol : dichloromethane 1:9), HPLC-MS (252.1 $[\text{M}]^+$, 275.2 $[\text{M} + \text{Na}]^+$, 527.2 $[2\text{M} + \text{Na}]^+$).

25

2*RS* 3-Cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid and (4*S*, 5*R*) 4-amino-5-methyl-dihydro-furan-3-one hydrochloride (31) were coupled as follows:

iso-Butyl chloroformate (7.15 mg, 0.0523 mmol) in dichloromethane (2 ml) and *N*-methyl morpholine (10.58 mg, 0.1046 mmol) in dichloromethane (2 ml) were
30 simultaneously added to a stirred solution of 2*RS* 3-Cyclohexyl-2-(furan-3-ylmethoxy)-propionic acid (12.00 mg, 0.048 mmol) in dichloromethane (2 ml) at –

15°C under argon over 5 minutes. The mixture was stirred at -15 °C for 15 minutes. A freshly prepared solution of (31) (7.21 mg, 0.048mmol) in dichloromethane (2 ml) and 4-methylmorpholine (5.29 mg, 0.0523 mmol) was then added dropwise. The mixture was then stirred at ambient temperature for 16 hours. The solvent was removed *in vacuo* to give a residue which was purified over silica gel eluting with a gradient of n-heptane : ethyl acetate 3:1. Desired fractions were combined and reduced *in vacuo* to leave (2*R*, 3*S*) 3-Cyclohexyl-2-(furan-3-ylmethoxy)-N-(2-methyl-4-oxo-tetrahydro-furan-3-yl)-propionamide as an off-white gum (6 mg, 35.77% from starting acid). TLC (single spot, *R*_f = 0.8, methanol : dichloromethane 1:9), analytical

¹H (CDCl₃ at 298K); 0.70-0.95 (2H, CH₂ (cyclohexyl), m), 1.02-1.25 (2H, CH₂ (cyclohexyl), m), 1.38 (3H, OCHCH₃, d, *J* = 6.12 Hz), 1.45-1.70 (8H, CH, CH₂ (cyclohexyl), m), 3.70-3.79 (1H, OCHCH₃, m), 3.80-4.08 (3H, OCHCONH, COCH₂O and NHCHCOCH₂, m), 4.14-4.23 (1H, COCH₂O, d, *J* = 17.20 Hz), 4.30-4.46 (2H, 3-Furan-CH₂O, m), 6.35 (1H, 2-Furan-CH, s), 6.76 (1H, NH, bs), 7.37 (2H, 4 and 5-Furan-CH, d, *J* = 1.67 Hz).

EXAMPLE A. Assays for Cysteine Protease Activity

The compounds of this invention may be tested in one of a number of literature based biochemical assays that are designed to elucidate the characteristics of compound inhibition. The data from these types of assays enables compound potency and the rates of reaction to be measured and quantified. This information, either alone or in combination with other information, would allow the amount of compound required to produce a given pharmacological effect to be determined.

General materials and methods

Unless otherwise stated, all general chemicals and biochemicals were purchased from either the Sigma Chemical Company, Poole, Dorset, U.K. or from Fisher Scientific UK, Loughborough, Leicestershire, U.K. Absorbance assays were carried out in flat-bottomed 96-well plates (Spectra; Greiner Bio-One Ltd., Stonehouse, Gloucestershire, U.K.) using a SpectraMax PLUS384 plate reader (Molecular Devices, Crawley, U.K.). Fluorescence high throughput assays were carried out in

either 384-well microtitre plates (Corning Costar 3705 plates, Fisher Scientific) or 96-well 'U' bottomed Microfluor W1 microtitre plates (Thermo Labsystems, Ashford, Middlesex, U.K.). Fluorescence assays were monitored using a SpectraMax Gemini fluorescence plate reader (Molecular Devices). For substrates
5 employing either a 7-amino-4-methylcoumarin (AMC) or a 7-amino-4-trifluoromethylcoumarin (AFC) fluorophore, assays were monitored at an excitation wavelength of 365 nm and an emission wavelength of 450 nm and the fluorescence plate reader calibrated with AMC. For substrates employing a 3-amino-benzoyl (Abz) fluorophore, assays were monitored at an excitation wavelength of 310 nm and
10 an emission wavelength of 445 nm; the fluorescence plate reader calibrated with 3-amino-benzamide (Fluka). Unless otherwise indicated, all the peptidase substrates were purchased from Bachem UK, St. Helens, Merseyside, UK. Substrates utilizing fluorescence resonance energy transfer methodology (*i.e.* FRET-based substrates) were synthesized at Incenta Limited using published methods (Atherton & Sheppard,
15 *Solid Phase Peptide Synthesis*, IRL Press, Oxford, U.K., 1989) and employed Abz (2-aminobenzoyl) as the fluorescence donor and 3-nitro-tyrosine [Tyr(NO₂)] as the fluorescence quencher (Meldal, M. and Breddam, K., *Anal. Biochem.*, 195, 141-147, 1991). Hydroxyethylpiperazine ethanesulfonate (HEPES), tris-hydroxymethyl aminomethane (tris) base, bis-tris-propane and all the biological detergents (*e.g.*
20 CHAPS, zwittergents, *etc.*) were purchased from CN Biosciences UK, Beeston, Nottinghamshire, U.K. Glycerol was purchased from Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, U.K. Stock solutions of substrate or inhibitor were made up to 10 mM in 100 % dimethylsulfoxide (DMSO) (Rathburns, Glasgow, U.K.) and diluted as appropriately required. In all cases the DMSO concentration in
25 the assays was maintained at less than 1% (vol./vol.).

Assay protocols were based on literature precedent (Table1; Barrett, A.J., Rawlings, N.D. and Woessner, J.F., 1998, *Handbook of Proteolytic Enzymes*, Academic Press, London and references therein) and modified as required to suit local assay
30 protocols. Enzyme was added as required to initiate the reaction and the activity, as judged by the change in fluorescence upon conversion of substrate to product, was monitored over time. All assays were carried out at 25±1°C.

Table 1. *The enzyme assays described herein were carried out according to literature precedents.*

Enzyme	Buffer	Substrate	Reference
Cathepsin B	I	Z-Phe-Arg-AMC	a, b
Cathepsin H	II	Bz-Phe-Val-Arg-AMC	a, b
Cathepsin L	I	Ac-Phe-Arg-AMC	b, c
Cathepsin S	I	Boc-Val-Leu-Lys-AMC	c,d
Caspase 1	III	Ac-Leu-Glu-His-Asp-AMC	e
Caspase 2	III	Z-Val-Asp-Val-Ala-Asp-AFC	f
Caspase 3	III	Ac-Asp-Glu-Val-Asp-AMC	g, h
Caspase 4	III	Suc-Tyr-Val-Ala-Asp-AMC	f
Caspase 5	III	Ac-Leu-Glu-His-Asp-AMC	
Caspase 6	III	Ac-Val-Glu-Ile-Asp-AMC	i, j, k
Caspase 7	III	Ac-Asp-Glu-Val- Asp-AMC	
Caspase 8	III	Ac-Ile-Glu-Thr-Asp-AMC	l
Caspase 9	III	Ac-Leu-Glu-His-Asp-AMC	
Caspase 10	III	Ac-Ile-Glu-Thr-Asp-AMC	
Cruzipain	IV	D-Val-Leu-Lys-AMC	m, n
CPB2.8ΔCTE	XI	Pro-Phe-Arg-AMC	q
<i>S. Aureus</i> Extracellular cysteine peptidase	I	Abz-Ile-Ala-Ala-Pro- Tyr(NO ₂)-Glu-NH ₂	o
Clostripain		Z-Gly-Gly-Arg-AMC	p
FMDV LP	V	Abz-Arg-Lys-Leu-Lys-Gly- Ala-Gly-Ser-Tyr(NO ₂)-Glu- NH ₂	r
Trypsin	VI	Z-Gly-Gly-Arg-AMC	s
Calpain μ	VII	Abz-Ala-Asn-Leu-Gly-Arg-Pro- Ala-Leu-Tyr(NO ₂)-Asp-NH ₂	t
Calpain m	VIII	Abz-Lys-Leu-Cys(Bzl)-Phe-Ser- Lys-Gln-Tyr(NO ₂)-Asp-NH ₂	t

Cathepsin K	IX	Z-Phe-Arg-AMC	u
Cathepsin X	X		v,w

I: 10 mM BTP, pH 6.5 containing 1 mM EDTA, 5 mM 2-mercaptoethanol and 1 mM CaCl₂

II: 10 mM BTP, pH 6.5 containing 1 mM EDTA, 142 mM NaCl, 1 mM DTT, 1 mM CaCl₂, 0.035 mM Zwittergent 3-16

III: 50mM HEPES pH 7.2, 10% Glycerol, 0.1% CHAPS, 142 mM NaCl, 1 mM EDTA, 5 mM DTT

IV: 100 mM sodium phosphate, pH 6.75 containing 1 mM EDTA and 10 mM L-cysteine

V: 50 mM trisacetate, pH 8.4 containing 1 mM EDTA, 10 mM L-cysteine and 0.25% (w/v) CHAPS

VI: 10 mM HEPES, pH 8.0 containing 5 mM CaCl₂

VII: 10 mM HEPES, pH 7.5 containing 2 mM 2-mercaptoethanol and 100 µM CaCl₂

VIII: 10 mM HEPES, pH 7.5 containing 2 mM 2-mercaptoethanol and 200 µM CaCl₂

IX: 100 mM sodium acetate; pH 5.5 containing 10 mM L-cysteine and 1 mM EDTA

X: 100 mM sodium acetate; pH 5.5 containing 10 mM L-cysteine; 0.05% (w/v) Brij 35 and 1 mM EDTA

XI: 100 mM sodium acetate; pH 5.5 containing 10 mM L-cysteine; 142 mM sodium chloride and 1 mM EDTA

^a Barrett, A.J., *Biochem. J.*, 187, 909-912, 1980

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^d Bromme, D., Steinert, ., Freibe, S., Fittkau, S., Wiederanders, B., and Kirschke, H., *Biochem. J.*, 264, 475-481, 1989

^e Rano, T.A., *et. al.*, *Chem. Biol.*, 4, 149, 1997

^f Talanian, R.V., *et. al.*, *J. Biol. Chem.*, 272, 9677, 1997

^g Lazebnik, Y.A., Kaufmann, S.H., Desnoyers, S., Poirer, G.G. and Earnshaw, W.C., *Nature.*, 371, 768-774, 1994

- ^h Han, Z., *et. al.*, *J. Biol. Chem.*, 272, 13432, 1997
- ⁱ Takahashi, A., *et. al.*, *PNAS*, 93, 8395, 1996
- ^j Martins, L.M., *et. al.*, *J. Biol. Chem.*, 272, 7421, 1997
- ^k Nagata, S., *Cell.*, 88, 355, 1997
- 5 ^l Harris, J.L., *et. al.*, *J. Biol. Chem.*, 273, 27364, 1998
- ^m Cazzulo, J.J., Cazzulo Franke, M.C., Martinez, J. and Franke de Cazzulo, B.M., *Biochim. Biophys. Acta.*, 1037, 186-191, 1990
- ⁿ Cazzulo, J.J., Bravo, M., Raimondi, A., Engstrom, U., Lindeberg, G. and Hellman, U., *Cell Mol. Biol.*, 42, 691-696, 1996
- 10 ^o Potempa, J., Dubin, A., Korzus, G. and Travis, J., *Biochem. J.*, 263, 2664-2667, 1988
- ^p Kembhavi, A.A., Buttle, D.J., Rauber, P. and Barrett, A.J., *FEBS Lett.*, 283, 277-280, 1991
- ^q Alves, L.C., *et. al.*, *Mol. Biochem. Parasitol.*, 116, 1-9, 2001.
- 15 ^r Guarné, *et.al.*, *J. Mol. Biol.*, 302, 1227-1240, 2000.
- ^s Halfon and Craik, (Barret, Rawlings and Woessner, eds.), in *Handbook of Proteolytic Enzymes*, Academic Press, London, 12-21, 1998.
- ^t Sasaki, *et. al.*, (1984), *J. Biol. Chem.*, 259, 12489-12494, 1984.
- ^u Bossard, M.J., *et. al.*, *J. Biol. Chem.*, 271, 12517-12524, 1996
- 20 ^v Santamaria, I., *et. al.*, *J. Biol. Chem.*, 273, 16816-16823, 1998
- ^w Klemencic, J, *et al.*, *Eur.J.Biochem.*, 267, 5404-5412, 2000

Trypanosoma cruzi cruzipain peptidase activity assays

- 25 Wild-type cruzipain, derived from *Trypanosoma cruzi* Dm28 epimastigotes, was obtained from Dr. Julio Scharfstein (Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil). Activity assays were carried out in 100 mM sodium phosphate, pH 6.75 containing 1 mM EDTA and 10 mM L-cysteine using 2.5 nM enzyme. Ac-Phe-Arg-AMC ($K_M^{app} \approx 12 \mu M$) and D-Val-Leu-Lys-AMC ($K_M^{app} \approx 4 \mu M$) were used as the substrates. Routinely, Ac-FR-AMC was used at a concentration equivalent to K_M^{app} and D-Val-Leu-Lys-AMC was used at a concentration of 25 μM . The rate of conversion of substrate to product was
- 30

derived from the slope of the increase in fluorescence monitored continuously over time.

Leishmania mexicana cysteine protease B (CPB) peptidase activity assays

5 Wild-type recombinant CPB without the C-terminal extension (*i.e.* CPB2.8 Δ CTE; Sanderson, S.J., *et al.*, *Biochem. J.*, 347, 383-388, **2000**) was obtained from Dr. Jeremy Mottram (Wellcome Centre for Molecular Parasitology, The Anderson College, University of Glasgow, Glasgow, U.K.). Activity assays were carried out in
10 100 mM sodium acetate; pH 5.5 containing 1 mM EDTA; 200 mM NaCl and 10 mM DTT (Alves, L.C., *et al.*, *Mol. Biochem. Parasitol.*, 116, 1-9, **2001**) using 0.25 nM enzyme. Pro-Phe-Arg-AMC ($K_M^{app} \approx 38\mu\text{M}$) was used as the substrate at a concentration equivalent to K_M^{app} . The rate of conversion of substrate to product was
15 time.

Cathepsin peptidase activity assays

Bovine cathepsin S, human cathepsin L, human cathepsin H and human cathepsin B
20 were obtained from CN Biosciences. Recombinant human cathepsin S, human cathepsin K and human cathepsin X were obtained from Dr. Boris Turk (Josef Stefan Institute, Ljubljana, Slovenia). Unless otherwise stated, all peptidase activity assays were carried out in 10 mM bis-tris-propane (BTP), pH 6.5 containing 1 mM EDTA, 5 mM 2-mercaptoethanol and 1 mM CaCl_2 . Human cathepsin H activity assays were
25 carried out in 10 mM BTP pH 6.5, 142 mM NaCl_2 , 1 mM CaCl_2 , 1 mM EDTA, 1 mM DTT, 0.035 mM Zwittergent 3-16. Human cathepsin K assays were carried out in 100 mM sodium acetate; pH 5.5 containing 20 mM L-cysteine and 1 mM EDTA (Bossard, M.J., *et al.*, *J. Biol. Chem.*, 271, 12517-12524, **1996**). Human cathepsin X assays were carried out in 100 mM sodium acetate; pH 5.5 containing 20 mM L-cysteine; 0.05% (w/v) Brij 35 and 1 mM EDTA (Santamaria, I., *et al.*, *J. Biol. Chem.*, 273, 16816-16823, **1998**; Klemencic, J, *et al.*, *Eur. J. Biochem.*, 267, 5404-5412, **2000**). The final enzyme concentrations used in the assays were 0.5 nM bovine cathepsin S, 1 nM cathepsin L, 0.1 nM cathepsin B, 0.25nM Cathepsin K; 1
30

nM cathepsin X and 10 nM cathepsin H. For the inhibition assays, the substrates used for cathepsin S, cathepsin L, cathepsin B, cathepsin K and cathepsin H were boc-Val-Leu-Lys-AMC ($K_M^{app} \approx 30 \mu M$), Ac-Phe-Arg-AMC ($K_M^{app} \approx 20 \mu M$), Z-Phe-Arg-AMC ($K_M^{app} \approx 40 \mu M$), Z-Leu-Arg-AMC ($K_M^{app} \approx 2 \mu M$); Bz-Phe-Val-Arg-AMC ($K_M^{app} \approx 150 \mu M$) respectively. In each case the substrate concentration used in each assay was equivalent to the K_M^{app} . The rate of conversion of substrate to product was derived from the slope of the increase in fluorescence monitored continuously over time.

10 Trypsin peptidase activity assays

Human pancreatic trypsin (iodination grade; CN Biosciences) activity assays were carried out in 10 mM HEPES, pH 8.0 containing 5 mM $CaCl_2$ using 0.1 nM trypsin. For the inhibition assays, Z-Gly-Gly-Arg-AMC ($K_M^{app} \approx 84 \mu M$) was used as the substrate at a concentration equivalent to K_M^{app} . The rate of conversion of substrate to product was derived from the slope of the increase in fluorescence monitored continuously over time.

Clostripain peptidase activity assays

Clostripain (Sigma) activity assays were carried out in 10 mM BTP, pH 6.5 containing 1 mM EDTA, 5 mM 2-mercaptoethanol and 1mM $CaCl_2$ using 0.3 nM enzyme. For the inhibition assays, Z-Gly-Gly-Arg-AMC ($K_M^{app} \approx 100 \mu M$) was used as the substrate at a concentration equivalent to K_M^{app} . The rate of conversion of substrate to product was derived from the slope of the increase in fluorescence monitored continuously over time.

Calpain peptidase activity assays

Calpain (human erythrocyte μ -calpain and porcine kidney m-calpain; CN Biosciences) activity assays were carried out in 10 mM HEPES, pH 7.5 containing 2 mM 2-mercaptoethanol and $CaCl_2$ using 25 nM of either enzyme (Sasaki, *et. al.*, *J. Biol. Chem.*, 259, 12489-12494, 1984). For μ -calpain inhibition assays, the buffer

contained 100 μM CaCl_2 and Abz-Ala-Asn-Leu-Gly-Arg-Pro-Ala-Leu-Tyr(NO_2)-Asp- NH_2 ($K_M^{\text{app}} \approx 20 \mu\text{M}$; Incenta Limited) was used as the substrate. For m-calpain inhibition assays, the assay buffer contained 200 μM CaCl_2 and Abz-Lys-Leu-Cys(Bzl)-Phe-Ser-Lys-Gln-Tyr(NO_2)-Asp- NH_2 ($K_M^{\text{app}} \approx 22 \mu\text{M}$; Incenta Limited) was used as the substrate. In both cases the substrate concentration employed in the assays was equivalent to the K_M^{app} . The rate of conversion of substrate to product was derived from the slope of the increase in fluorescence monitored continuously over time.

10 Extracellular *S. aureus* V8 cysteine peptidase (staphylopain) peptidase activity assays

S. aureus V8 was obtained from Prof. S. Arvidson, Karolinska Institute, Stockholm, Sweden. Extracellular *S. aureus* V8 cysteine peptidase (staphylopain) activity assays were carried out using partially purified *S. aureus* V8 culture supernatant (obtained from Dr. Peter Lambert, Aston University, Birmingham, U.K.). Activity assays were carried out in 10 mM BTP, pH 6.5 containing 1 mM EDTA, 5 mM 2-mercaptoethanol and 1mM CaCl_2 using two-times diluted partially purified extract. For the inhibition assays, Abz-Ile-Ala-Ala-Pro-Tyr(NO_2)-Glu- NH_2 ($K_M^{\text{app}} \approx 117 \mu\text{M}$; Incenta Limited) was used as the substrate at a concentration equivalent to K_M^{app} . The rate of conversion of substrate to product was derived from the slope of the increase in fluorescence monitored continuously over time.

Foot-and-mouth disease leader peptidase (FMDV-LP) activity assays

25 Recombinant wild-type FMDV-LP was obtained from Dr. Tim Skern (Institut für Medizinische Biochemie, Abteilung für Biochemie, Universität Wien, Wien, Austria). Activity assays were carried out in 50 mM trisacetate, pH 8.4 containing 1 mM EDTA, 10 mM L-cysteine and 0.25% (w/v) CHAPS using 10 nM enzyme. For the inhibition assays, Abz-Arg-Lys-Leu-Lys-Gly-Ala-Gly-Ser-Tyr(NO_2)-Glu- NH_2 ($K_M^{\text{app}} \approx 51 \mu\text{M}$, Incenta Limited) was used as the substrate at a concentration equivalent to K_M^{app} . The rate of conversion of substrate to product was derived from the slope of the increase in fluorescence monitored continuously over time.

Caspase peptidase activity assays

Caspases 1-10 were obtained from CN Biosciences or BioVision Inc. (Mountain View, CA, USA) and all assays were carried out in 50mM HEPES; pH 7.2, 10% (v/v) glycerol, 0.1% (w/v) CHAPS, 142 mM NaCl, 1 mM EDTA, 5 mM dithiothreitol (DTT) using 0.1-1 U per assay. For caspase 1, Ac-Leu-Glu-His-Asp-AMC was used as the substrate; for caspase 2, Z-Val-Asp-Val-Ala-Asp-AFC was used as the substrate; for caspase 3, Ac-Asp-Glu-Val-Asp-AMC was used as the substrate; for caspase 4, Suc-Tyr-Val-Ala-Asp-AMC was used as the substrate; for caspase 5, Ac-Leu-Glu-His-Asp-AMC was used as the substrate; for caspase 6, Ac-Val-Glu-Ile-Asp-AMC was used as the substrate; for caspase 7, Ac-Asp-Glu-Val-Asp-AMC was used as the substrate; for caspase 8, Ac-Ile-Glu-Thr-Asp-AMC was used as the substrate; for caspase 9, Ac-Leu-Glu-His-Asp-AMC was used as the substrate; for caspase 10, Ac-Ile-Glu-Thr-Asp-AMC was used as the substrate (Nicholson, D.W. and Thornberry, N.A., *TIBS*, 22, 299-306, 1997; Stennicke, H.R. and Salvesen, G.S., *J. Biol. Chem.*, 272(41), 25719-25723, 1997; Talanian, R.V., *et al.*, *J. Biol. Chem.*, 272(15), 9677-9682, 1997; Wolf, B.B. and Green, D.R., *J. Biol. Chem.*, 274(29), 20049-20052, 1999). The rate of conversion of substrate to product was derived from the slope of the increase in fluorescence monitored continuously over time.

Measurement of the apparent macroscopic binding (Michaelis) constants (K_M^{app}) for substrates

The apparent macroscopic binding constant (K_M^{app}) for each substrate was calculated, from the dependence of enzyme activity as a function of substrate concentration. The observed rates were plotted on the ordinate against the related substrate concentration on the abscissa and the data fitted by direct regression analysis (Prism v 3.02; GraphPad, San Diego, USA) using Equation 1 (Cornish-Bowden, A. *Fundamentals of enzyme kinetics* Portland Press; 1995, 93-128.).

$$v_i = \frac{V_{\max}^{app} \cdot [S_o]}{[S_o] + K_M^{app}} \quad (1)$$

In Equation 1 ' v_i ' is the observed initial rate, ' V_{\max}^{app} ' is the observed maximum activity at saturating substrate concentration, ' K_M^{app} ' is the apparent macroscopic binding (Michaelis) constant for the substrate, ' $[S_o]$ ' is the initial substrate concentration.

Measurement of the inhibition constants

The apparent inhibition constant (K_i) for each compound was determined on the basis that inhibition was reversible and occurred by a pure-competitive mechanism. The K_i values were calculated, from the dependence of enzyme activity as a function of inhibitor concentration, by direct regression analysis (Prism v 3.02) using Equation 2 (Cornish-Bowden, A., 1995).

$$v_i = \frac{V_{\max}^{app} \cdot [S]}{[S] + \{K_M^{app} \cdot ([I] / K_i)\}} \quad (2)$$

In Equation 2 ' v_i ' is the observed residual activity, ' V_{\max}^{app} ' is the observed maximum activity (i.e. in the absence of inhibitor), ' K_M^{app} ' is the apparent macroscopic binding (Michaelis) constant for the substrate, ' $[S]$ ' is the initial substrate concentration, ' K_i ' is the apparent dissociation constant and ' $[I]$ ' is the inhibitor concentration.

In situations where the apparent dissociation constant (K_i^{app}) approached the enzyme concentrations, the K_i^{app} values were calculated using a quadratic solution in the form described by Equation 3 (Morrison, J.F. *Trends Biochem. Sci.*, 7, 102-105, 1982; Morrison, J.F. *Biochim. Biophys. Acta*, 185, 269-286, 1969; Stone, S.R. and Hofsteenge, J. *Biochemistry*, 25, 4622-4628, 1986).

$$v_i = \frac{F\{E_o - I_o - K_i^{app} + \sqrt{(E_o - I_o - K_i^{app})^2 + 4 \cdot K_i^{app} \cdot E_o}\}}{2} \quad (3)$$

$$K_i^{app} = K_i(1 + [S_o] / K_M^{app}) \quad (4)$$

In Equation 3 'v_i' is the observed residual activity, 'F' is the difference between the maximum activity (*i.e.* in the absence of inhibitor) and minimum enzyme activity, 'E_o' is the total enzyme concentration, 'K_i^{app}' is the apparent dissociation constant and 'I_o' is the inhibitor concentration. Curves were fitted by non-linear regression analysis (Prism) using a fixed value for the enzyme concentration. Equation 4 was used to account for the substrate kinetics, where 'K_i' is the inhibition constant, '[S_o]' is the initial substrate concentration and 'K_M^{app}' is the apparent macroscopic binding (Michaelis) constant for the substrate (Morrison, 1982).

The second-order rate of reaction of inhibitor with enzyme

Where applicable, the concentration dependence of the observed rate of reaction (*k*_{obs}) of each compound with enzyme was analysed by determining the rate of enzyme inactivation under pseudo-first order conditions in the presence of substrate (Morrison, J.F., *TIBS*, 102-105, 1982; Tian, W.X. and Tsou, C.L., *Biochemistry*, 21, 1028-1032, 1982; Morrison, J.F. and Walsh, C.T., from Meister (Ed.), *Advances in Enzymol.*, 61, 201-301, 1988; Tsou, C.L., from Meister (Ed.), *Advances in Enzymol.*, 61, 381-436, 1988;). Assays were carried out by addition of various concentrations of inhibitor to assay buffer containing substrate. Assays were initiated by the addition of enzyme to the reaction mixture and the change in fluorescence monitored over time. During the course of the assay less than 10% of the substrate was consumed.

$$F = v_s t + \frac{(v_o - v_s) [1 - e^{-(k_{obs} t)}]}{k_{obs}} + D \quad (5)$$

The activity fluorescence progress curves were fitted by non-linear regression analysis (Prism) using Eq. 5 (Morrison, 1969; Morrison, 1982); where 'F' is the fluorescence response, 't' is time, 'v_o' is the initial velocity, 'v_s' is the equilibrium steady-state velocity, 'k_{obs}' is the observed pseudo first-order rate constant and 'D' is

the intercept at time zero (*i.e.* the ordinate displacement of the curve). The second order rate constant was obtained from the slope of the line of a plot of k_{obs} versus the inhibitor concentration (*i.e.* $k_{obs}/[I]$). To correct for substrate kinetics, Eq. 6 was used, where ' $[S_o]$ ' is the initial substrate concentration and ' K_M^{app} ' is the apparent macroscopic binding (Michaelis) constant for the substrate.

$$k_{inact} = \frac{k_{obs} (1 + [S_o] / K_M^{app})}{[I]} \quad (6)$$

Compounds of the invention were tested by the above described assays and observed to exhibit cruzipain inhibitory activity or inhibitory activity against an alternative CA C1 cysteine protease with an *in vitro* K_i inhibitory constant of less than or equal to 100 μ M. Exemplary inhibition data for a number of example compounds of the invention are given in table 2.

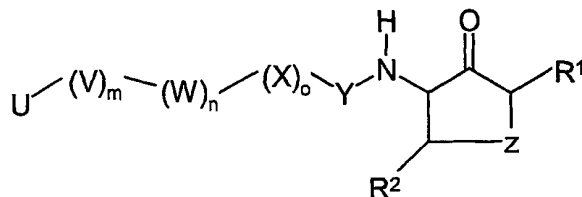
Table 2. Exemplary inhibition data (K_i expressed as μ M).

EXAMPLE N°	Cruzipain	Bovine Cathepsin S	Human Cathepsin L	Human Cathepsin K
22	<5	>50	>100	>5
46	>10	>20	>5	<1
49	>100	>100	<10	>100
4	>100	<10	>100	>100

CLAIMS

1. A compound according to general formula (I):

5



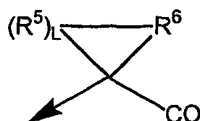
(I)

wherein: $R^1 = C_{0-7}$ -alkyl (when $C = 0$, R^1 is simply hydrogen), C_{3-6} -cycloalkyl or $Ar-C_{0-7}$ -alkyl (when $C = 0$, R^1 is simply an aromatic moiety Ar);

10

$R^2 = C_{1-7}$ -alkyl, C_{3-6} -cycloalkyl or $Ar-C_{0-7}$ -alkyl;

$Y = CHR^3-CO$ or CR^3R^4-CO where R^3 and R^4 are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl and $Ar-C_{0-7}$ -alkyl, or Y represents



15

where L is a number from one to four and R^5 and R^6 are independently chosen from CR^7R^8 where R^7 and R^8 are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl, $Ar-C_{0-7}$ -alkyl or halogen; and for each R^5 and R^6 either R^7 or R^8 (but not both R^7 and R^8) may additionally be chosen from $O-C_{0-7}$ -alkyl, $O-C_{3-6}$ -cycloalkyl, $O-Ar-C_{0-7}$ -alkyl, $S-C_{0-7}$ -alkyl, $S-C_{3-6}$ -cycloalkyl, $S-Ar-C_{0-7}$ -alkyl, $NH-C_{0-7}$ -alkyl, $NH-C_{3-6}$ -cycloalkyl, $NH-Ar-C_{0-7}$ -alkyl, $N-(C_{0-7}-alkyl)_2$, $N-(C_{3-6}-cycloalkyl)_2$, and $N-(Ar-C_{0-7}-alkyl)_2$;

20

25

$(X)_o = CR^9R^{10}$, where R^9 and R^{10} are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl and $Ar-C_{0-7}$ -alkyl and o is a number from zero to three;

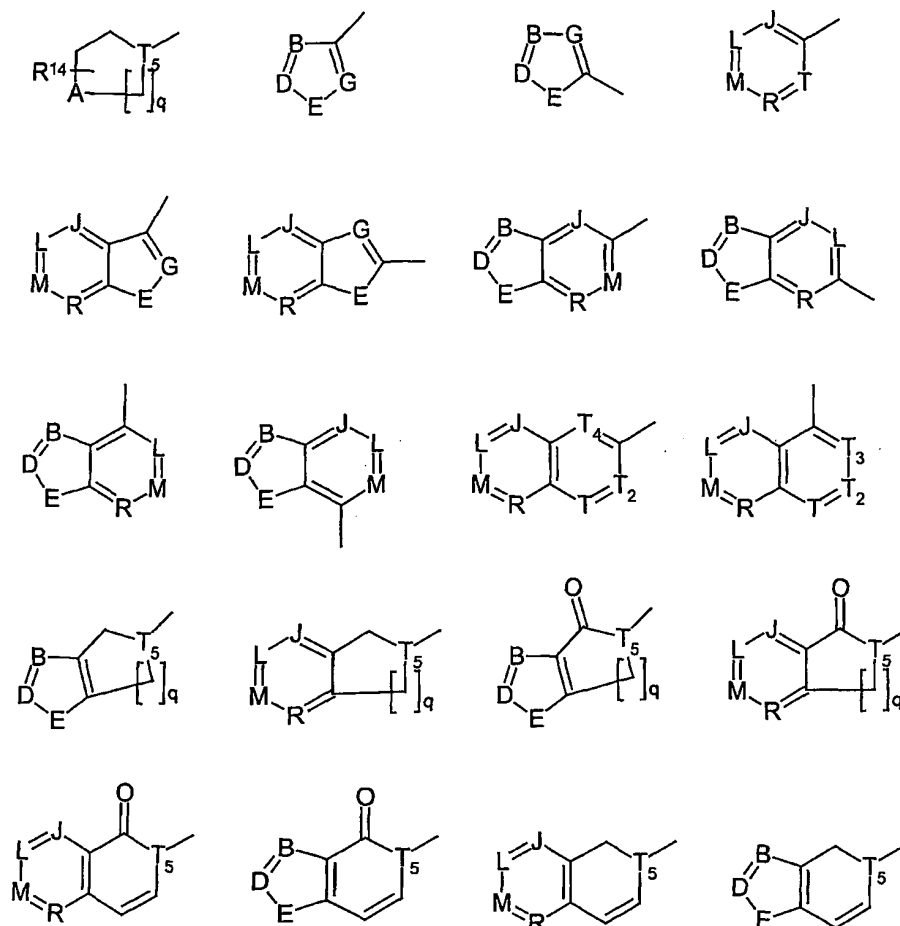
5 $(W)_n = O, S, C(O), S(O)$ or $S(O)_2$ or, when o is one or greater, NR^{11} , where R^{11} is chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl and $Ar-C_{0-7}$ -alkyl and n is zero or one;

10 $(V)_m = C(O), C(S), S(O), S(O)_2, S(O)_2NH, OC(O), NHC(O), NHS(O), NHS(O)_2, OC(O)NH, C(O)NH$ or $CR^{12}R^{13}$, where R^{12} and R^{13} are independently chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl, $Ar-C_{0-7}$ -alkyl and m is a number from zero to three, provided that when m is greater than one, $(V)_m$ contains a maximum of one carbonyl or sulphonyl group;

15 $Z = O$ (in which case compounds of general formula (I) may be named as (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amides),
 S (in which case compounds of general formula (I) may be named as (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amides), or
 CH_2 (in which case compounds of general formula (I) may be
 20 named as (2-alkyl-5-oxocyclopentyl)amides);

$U =$ a stable 5- to 7-membered monocyclic or a stable 8- to 11-membered bicyclic ring which is either saturated or unsaturated and which includes zero to four heteroatoms (as detailed below):

25



wherein R^{14} is chosen from:

5

C_{0-7} -alkyl, C_{3-6} -cycloalkyl, Ar- C_{0-7} -alkyl, halogen, O- C_{0-7} -alkyl, O- C_{3-6} -cycloalkyl, O-Ar- C_{0-7} -alkyl, S- C_{0-7} -alkyl, S- C_{3-6} -cycloalkyl, S-Ar- C_{0-7} -alkyl, NH- C_{0-7} -alkyl, NH- C_{3-6} -cycloalkyl, NH-Ar- C_{0-7} -alkyl, N-(C_{0-7} -alkyl)₂, N-(C_{3-6} -cycloalkyl)₂ and N-(Ar- C_{0-7} -alkyl)₂;

10

A is chosen from:

CH_2 , CHR^{14} , O, S and NR^{15} , where R^{14} is as defined above and where R^{15} is chosen from C_{0-7} -alkyl, C_{3-6} -cycloalkyl and Ar- C_{0-7} -alkyl;

15

B, D and G are independently chosen from:

CR^{14} , where R^{14} is as defined above, or N;

E is chosen from:

5 CH_2 , CHR^{14} , O, S and NR^{15} , where R^{14} and R^{15} are defined as above;

J, L, M, R, T, T_2 , T_3 and T_4 are independently chosen from:

CR^{14} and N, where R^{14} is as defined above;

10 T_5 is chosen from:

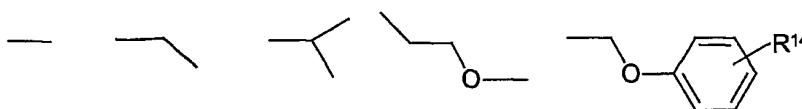
CH or, only when $m + n + o \geq 1$, T_5 may additionally be N;

q is a number from one to three, thereby defining a 5-, 6- or 7-membered ring.

15

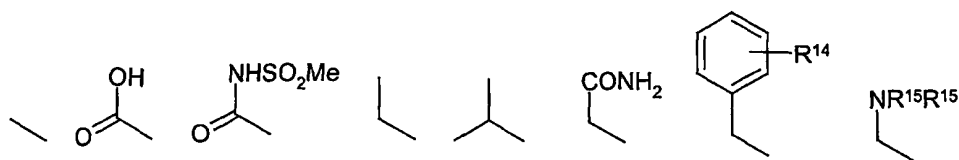
2. A compound as claimed in claim 1 wherein R^1 comprises C_{0-7} -alkyl or Ar- C_{0-7} -alkyl.

3. A compound as claimed in claim 2 wherein R^1 is selected from hydrogen or
20 one of the following moieties:



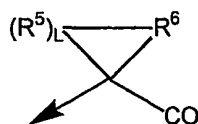
4. A compound as claimed in any one of claims 1 to 3 wherein R^2 is C_{1-7} -alkyl or
25 Ar- C_{0-7} -alkyl.

5. A compound as claimed in claim 4 wherein R^2 is selected from one of the following moieties:



wherein R^{14} and R^{15} are as defined previously.

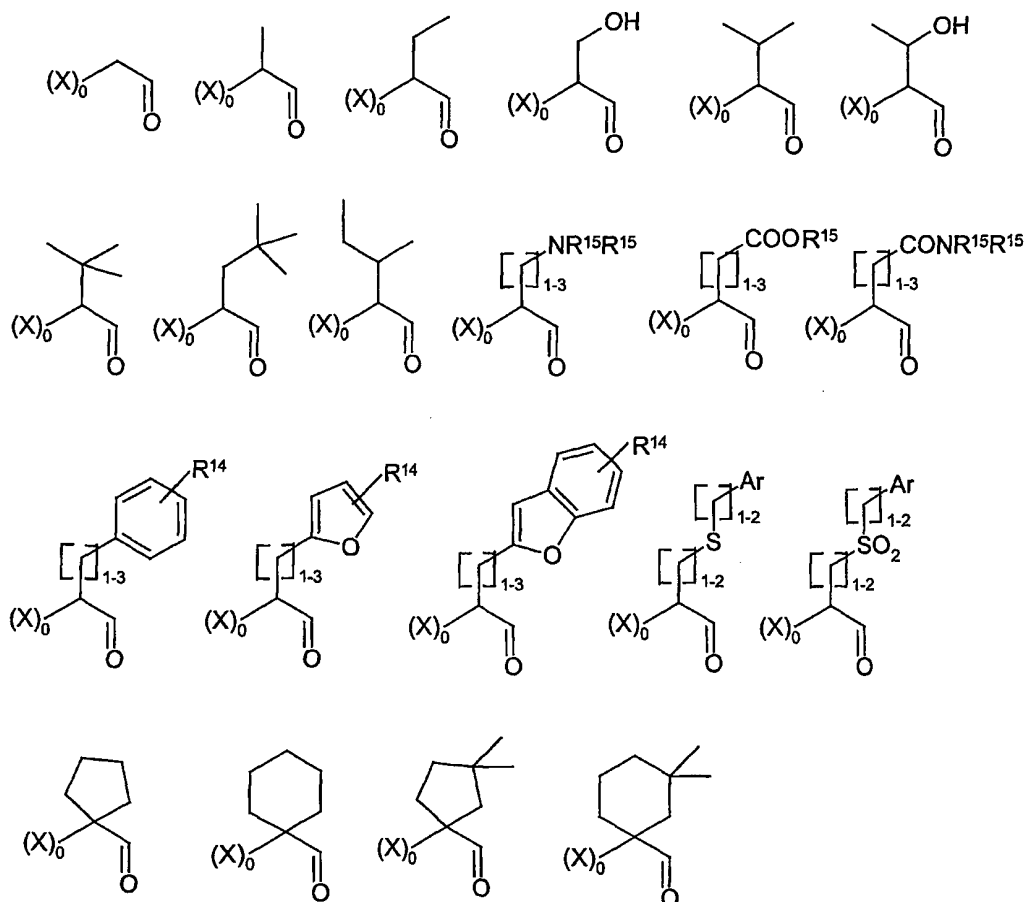
- 5 6. A compound as claimed in any one of claims 1 to 5 in which Z represents an oxygen atom.
7. A compound as claimed in any one of claims 1 to 6 wherein Y is CHR^4CO where R^4 is selected from C_{0-7} -alkyl or Ar-C_{0-7} -alkyl; or wherein Y comprises a
- 10 group:



where R^5 and R^6 are each CR^7R^8 and each R^7 and R^8 is, independently, selected from C_{0-7} -alkyl or Ar-C_{0-7} -alkyl.

15

8. A compound as claimed in any one of claims 1 to 6, wherein Y is CHR^4CO where R^4 is selected from C_{3-6} -cycloalkyl.
9. A compound as claimed in claim 1 wherein Y is selected from one of the
- 20 following moieties:

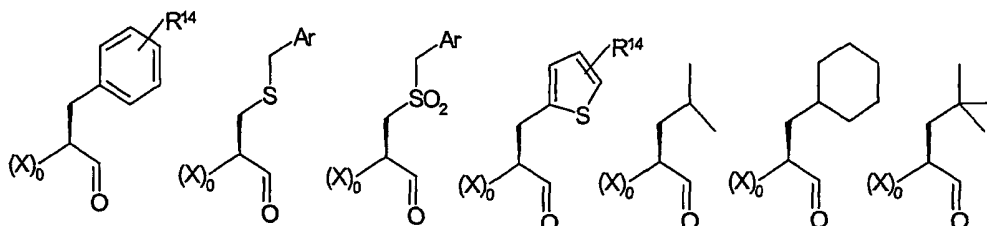


wherein R^{14} and R^{15} and Ar are as defined previously.

10. A compound as claimed in any one of claims 1 to 6, wherein Y is CHR^4CO where R^4 is $Ar-CH_2-$, where the aromatic ring is an optionally substituted phenyl or monocyclic heterocycle.
11. A compound as claimed in any one of claims 1 to 6, wherein Y is CHR^4CO where R^4 represents a simple branched alkyl group or a straight heteroalkyl chain.
12. A compound as claimed in any one of claims 1 to 6, wherein Y is CHR^4CO where the R^4 group comprises cyclohexylmethyl.

-133-

13. A compound as claimed in any one of claims 1 to 6, wherein Y is selected from the following:

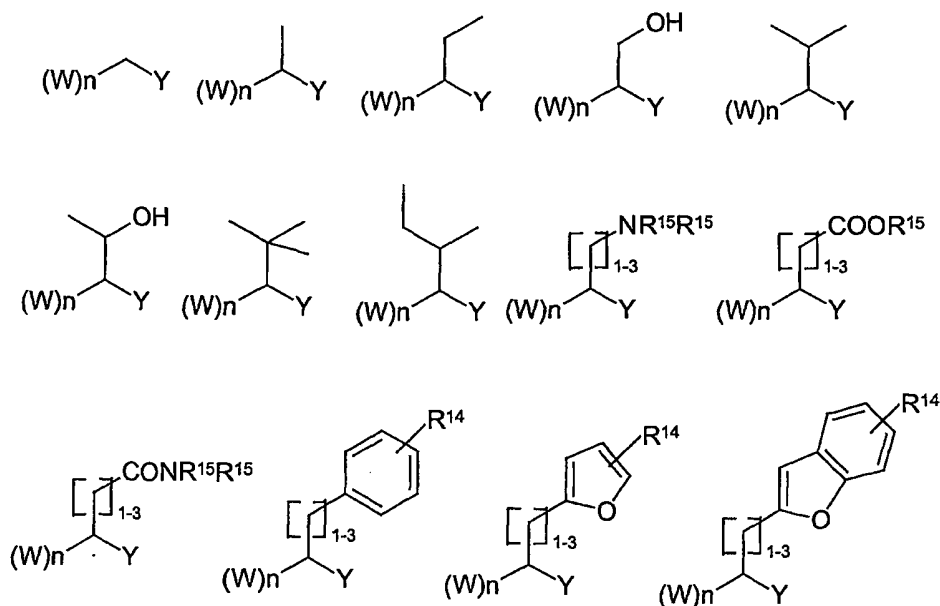


wherein R^{14} and Ar are as defined previously.

5

14. A compound as claimed in any one of claims 1 to 13 wherein, in the group $(X)_o$, X is CR^8R^9 and each of R^9 and R^{10} is selected from C_{0-7} -alkyl or Ar- C_{0-7} -alkyl

15. A compound as claimed in any one of claims 1 to 14, wherein $(X)_o$ is one of the following moieties:

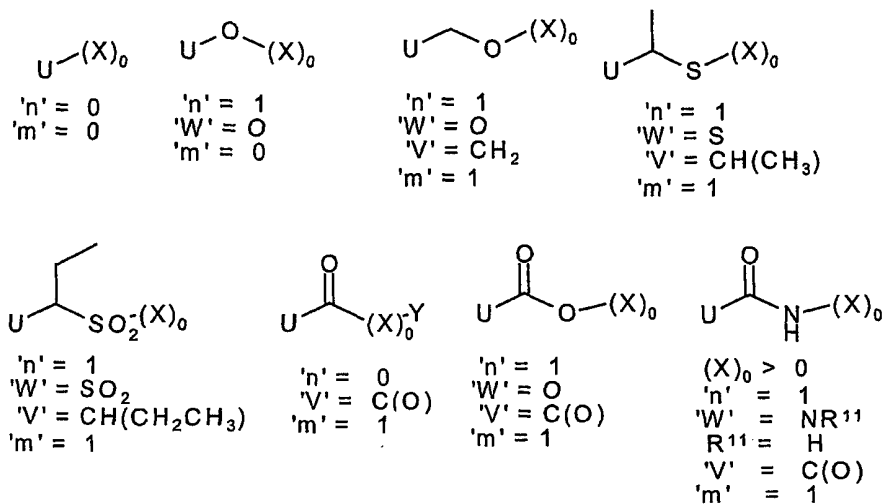


wherein R^{14} and R^{15} are as defined previously.

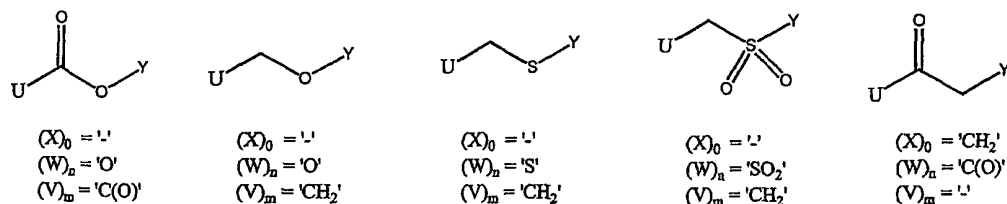
15

16. A compound as claimed in any one of claims 1 to 14, wherein $(X)_o$ a simple alkyl group and where $o = 0$ or 1 .
17. A compound as claimed in any one of claims 1 to 16 wherein, in the group
5 $(W)_n$:
W is O, S, SO_2 , SO, C(O) or when o is one or greater, NR^{11} , where R^{11} is C_{0-4} -alkyl; and n is 0 or 1.
18. A compound as claimed in any one of claims 1 to 16 wherein, in the group
10 $(W)_n$:
W is O, S, SO_2 , C(O) and n is 0 or 1.
19. A compound as claimed in any one of claims 1 to 18 wherein, in the group
 $(V)_m$:
15 V is C(O), C(O)NH or CHR^{13} , where R^{13} is C_{0-4} -alkyl; and
m is 0 or 1.
20. A compound as claimed in any one of claims 1 to 19 wherein the combination
 $(V)_m$ and $(W)_m$ is one of the following:

-135-

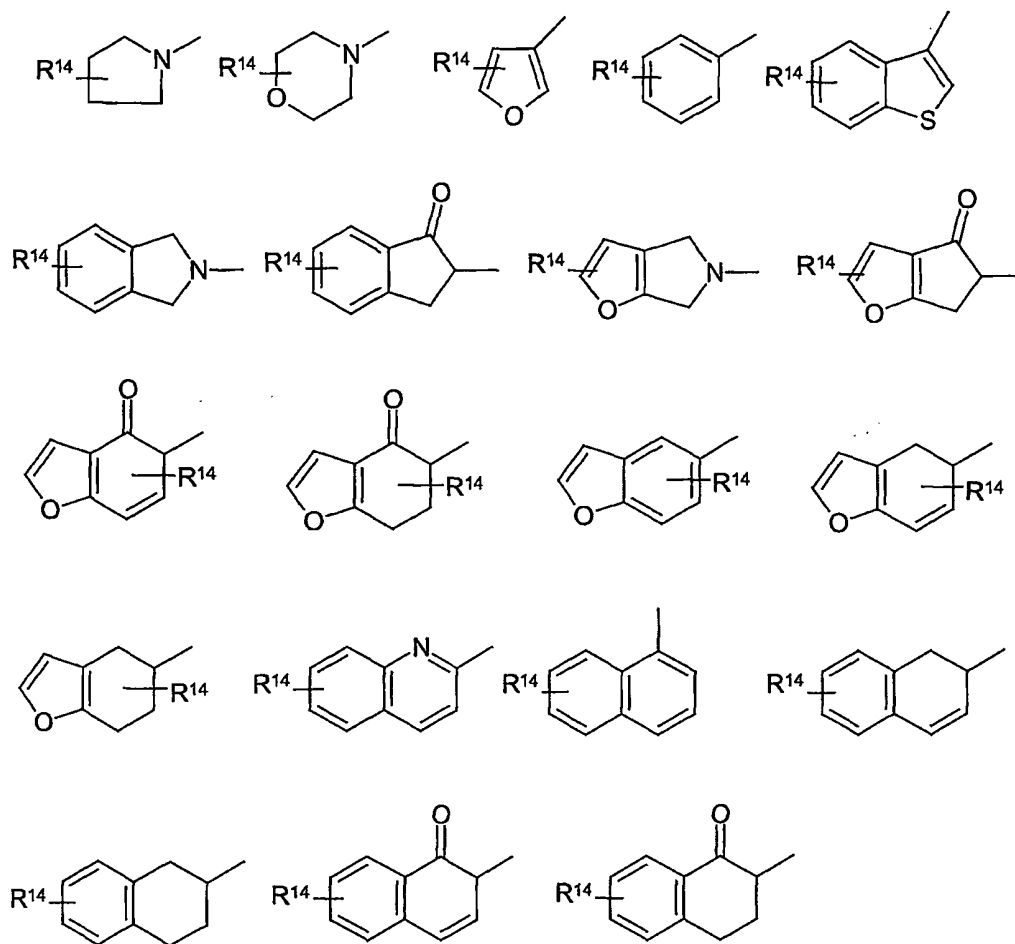


21. A compound as claimed in any one of claims 1 to 19 wherein the combination (X)₀, (V)_m and (W)_n is one of the following:



22. A compound as claimed in any one of claims 1 to 21 wherein U comprises an optionally substituted 5- or 6-membered saturated or unsaturated heterocycle or an optionally substituted saturated or unsaturated 9- or 10-membered heterocycle.

23. A compound as claimed in claim 22 wherein U comprises one of the following:



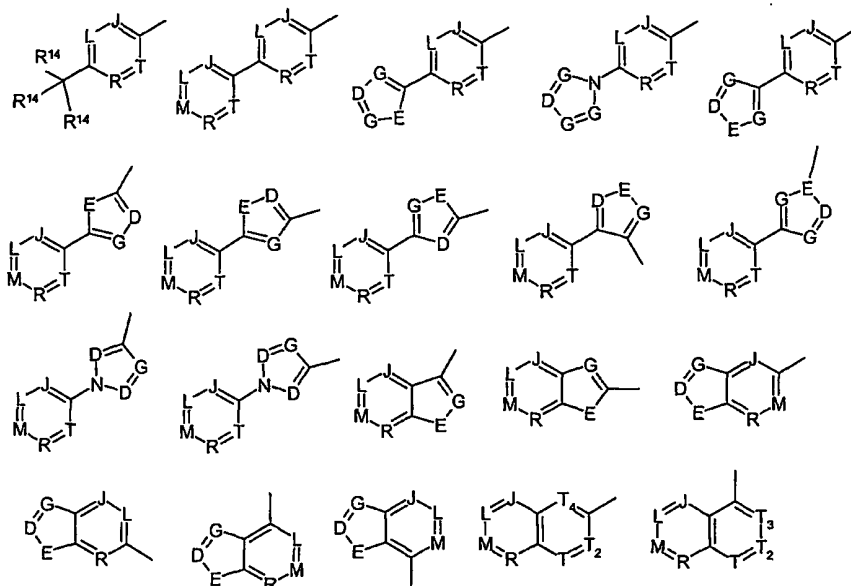
5 wherein R^{14} is as defined previously.

24. A compound as claimed in any one of claims 1 to 21 wherein U comprises a bulky alkyl or aryl group at the para position of an aryl Ar.

10 25. A compound as claimed in any one of claims 1 to 21 wherein U comprises a meta or para-biaryl Ar-Ar, where Ar is as previously defined.

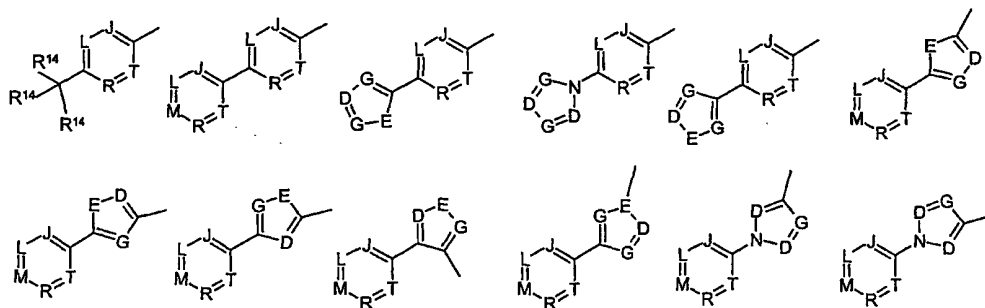
26. A compound as claimed in any one of claims 1 to 21 wherein U comprises a 6,6 or 6,5 or 5,6-fused aromatic ring.

27. A compound as claimed in any one of claims 1 to 21, wherein U represents a group:



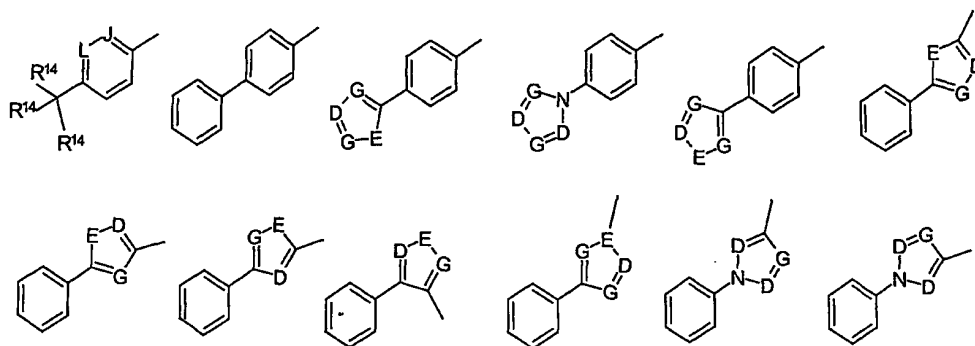
5 wherein R¹⁴, D, E, G, J, L, M, R, T, T₂, T₃ and T₄ are as defined previously.

28. A compound as claimed in any one of claims 1 to 21, wherein U represents a group



10 wherein R¹⁴, D, E, G, J, L, M, R and T are as defined previously.

29. A compound as claimed in any one of claims 1 to 21, wherein U represents a group



wherein R¹⁴, D, E, G, J and L are as defined previously.

5

30. A method of validating a known or putative cysteine protease inhibitor as a therapeutic target, the method comprising:

- (a) assessing the *in vitro* binding of a compound as claimed in any one of claims 1 to 29 to an isolated known or putative cysteine protease, providing a measure of 'potency'; and optionally, one or more of the steps of:
 - (b) assessing the binding of a compound as claimed in any one of claims 1 to 29 to closely related homologous proteases of the target and general house-keeping proteases (e.g. trypsin) to provides a measure of 'selectivity';
 - (c) monitoring a cell-based functional marker of a particular cysteine protease activity, in the presence of a compound as claimed in any one of claims 1 to 29; and
 - (d) monitoring an animal model-based functional marker of a particular cysteine protease activity, in the presence of a compound as claimed in any one of claims 1 to 29.

31. The use of a compound as claimed in any one of claims 1 to 29 in the validation of a known or putative cysteine protease inhibitor as a therapeutic target.

32. A compound as claimed in any one of claims 1 to 29 for use in medicine, especially for preventing or treating diseases in which the disease pathology may be modified by inhibiting a cysteine protease.

5

33. The use of a compound as claimed in any one of claims 1 to 29 in the preparation of a medicament for preventing or treating diseases in which the disease pathology may be modified by inhibiting a cysteine protease.

10 34. A compound as claimed in any one of claims 1 to 29 for use in preventing or treating Chagas' disease.

35. A compound as claimed in any one of claims 24 to 29 for use in preventing or treating Chagas' disease.

15

36. The use of a compound as claimed in any one of claims 1 to 29 in the preparation of a medicament for preventing or treating Chagas' disease.

20 37. The use of a compound as claimed in any one of claims 24 to 29 in the preparation of a medicament for preventing or treating Chagas' disease.

38. A pharmaceutical or veterinary composition comprising one or more compounds as claimed in any one of claims 1 to 29 and a pharmaceutically or veterinarily acceptable carrier.

25

39. A process for the preparation of a pharmaceutical or veterinary composition as claimed in claim 38, the process comprising bringing the active compound(s) into association with the carrier, for example by admixture.

30

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(81) Designated States (national): AE, AG, AL, AM, AT, AU,
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CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
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LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
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(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,

Declarations under Rule 4.17:

— as to the identity of the inventor (Rule 4.17(i)) for the fol-
lowing designations AE, AG, AL, AM, AT, AU, AZ, BA, BB,
BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK,
DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ,
OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO
patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG,
ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU,
TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES,
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patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,
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— as to applicant's entitlement to apply for and be granted
a patent (Rule 4.17(ii)) for the following designations AE,
AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA,
CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES,
FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG,
MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU,
SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG,
UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS,
MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent
(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,
MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI,
CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— of inventorship (Rule 4.17(iv)) for US only

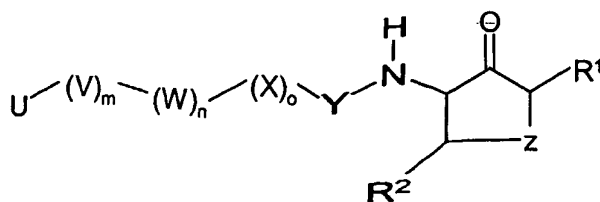
Published:

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3 October 2002

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: **INHIBITORS OF CRUZIPAIN AND OTHER CYSTEINE PROTEASES**



(I)

(57) Abstract: Compounds of
general formula (I): wherein R¹,
R², Y, (X)_o, (W)_n, (V)_m, Z and U
are as defined in the specification,
are inhibitors of cruzipain and
other cysteine protease inhibitors
and are useful as therapeutic
agents, for example in Chagas'
disease, or for validating
therapeutic target compounds.

WO 02/057248 A3

INTERNATIONAL SEARCH REPORT

International Application No

PC1/GB 02/00188

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D307/32 C07D307/38 C07D405/12 A61K31/5377 A61K31/473
A61K31/341 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, WPI Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 50533 A (FENWICK ASHLEY EDWARD ;GRIBBLE ANDREW D (GB); SMITHKLINE BEECHAM P) 12 November 1998 (1998-11-12) cited in the application abstract; claims page 4 -page 12; examples ---	1-29, 32, 33, 38, 39
X	WO 99 53039 A (SMITHKLINE BEECHAM CORP ;TOMASZEK THADDEUS ANTHONY (US); TEW DAVID) 21 October 1999 (1999-10-21) cited in the application page 2 abstract; claims page 6, line 5 - line 8 page 39 -page 42; example 11 --- -/--	1-29, 32, 33, 38, 39

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

9 July 2002

Date of mailing of the international search report

17/07/2002

Name and mailing address of the ISA

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Authorized officer

Paidsdor, B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00188

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 29408 A (GRIBBLE ANDREW D ;SMITHKLINE BEECHAM PLC (GB); WITHERINGTON JASON) 25 May 2000 (2000-05-25) cited in the application abstract; claims; examples 1-3 ---	1-29, 32, 33, 38, 39
X	WO 00 69855 A (TAYLOR STEVEN ;MEDIVIR UK LTD (GB); QUIBELL MARTIN (GB); PEPTIMMUN) 23 November 2000 (2000-11-23) cited in the application abstract; claims 1,12-16 ---	1-29, 32, 33, 38, 39
X	WO 98 05336 A (LO CASTRO STEPHEN ;MARQUIS ROBERT W JR (US); SMITHKLINE BEECHAM CO) 12 February 1998 (1998-02-12) cited in the application page 25, line 31 - line 34 abstract; claims -----	1-29, 32, 33, 38, 39

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-29 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds of formula (I) of claim 1 wherein Z has the meaning oxygen. All examples of the present application are (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amides, and not even a general method for preparing the compounds of formula (I) with Z having the meaning S or CH₂ is indicated in the application. It is the teaching of the present application how to provide (2-alkyl-4-oxo-tetrahydrofuran-3-yl)amide-derivatives (see pages 31 - 44 and schemes 1 - 10) and is completely silent on the preparation of (2-alkyl-4-oxo-tetrahydrothiophen-3-yl)amides or (2-alkyl-5-oxo-cyclopentyl)amides. Since the chemistry of these different "building blocks" (cf. page 31, lines 1-3) can be completely different, a person of ordinary skill in the art could not provide all compounds falling within the ambit of claims 1-29 without undue (experimental) effort.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/00188

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00188

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00188

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9805336	A	SK 88997 A3	06-05-1998
		TR 9700560 T1	21-11-1997
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